MODIFYING *ACACIA KOA* PHENOTYPE IN NURSERY CULTURE THROUGH CONTAINER SELECTION AND NITROGEN HARDENING TO PROMOTE SURVIVAL AND GROWTH IN THE FIELD

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Abstract

After centuries of habitat loss, the distribution of *Acacia koa* (koa) has largely been relegated to high-elevation, fragmented populations. In addition to being one of the most valuable trees in the world, koa provides critical habitat for endangered plant and animal species and is revered in Hawaiian culture. Invasive plant competition, animal browsing, drought, and climate change challenge establishment of koa seedlings. Climate change induced decreases in available soil moisture in conjunction with increases in solar radiation and temperature will greatly stress outplanted seedlings. Ensuring the survival of nursery-grown seedlings on sites that contain limited soil-moisture necessitates the employment of horticultural techniques in the nursery that modify morphological and physiological attributes of field-bound seedlings. Nutrition and container-type influence the survival and growth of outplanted seedlings. The root-to-shoot ratio (R:S) is a standard measure of seedling morphology, which is commonly used to predict drought avoidance potential and establishment success. High-quality seedlings have shoots that are not so large as to have a transpiration requirement that cannot be met by the roots at the time of planting. Nitrogen hardening is a horticultural technique in which the amount of applied nitrogen is reduced in the weeks prior to outplanting to decrease height and shoot growth, while increasing root growth and R:S. Deeper containers train roots to soil depths that can contain increased water, while air-pruning containers create a fibrous root system with an increased quantity of root tips. To test the efficacy of Nitrogen hardening koa for outplanting, seedlings were grown for 13 weeks in Deepot™ (25.4 cm deep) and RootMaker® (10.2 cm deep) containers (both 410 cm^3), with and without Nitrogen hardening. Seedlings were outplanted into a field site in the Northwestern Koʻolau Mountains in January, 2016. At the end of nursery culture, Nitrogen hardened and Deepot™ seedlings exhibited a significantly increased R:S. Nitrogen hardening did not confer survival or growth benefits to seedlings in the field. All seedlings exhibited a high survival rate 8 months after planting (>95%). Container-type was the most influential factor, with Deepot™ containers demonstrating

a significantly increased height (+9.4%) and root-collar diameter (+12.5%) after 8 months of field growth compared to RootMaker® containers.

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CHAPTER 1

INTRODUCTION

Origins of Acacia koa

Acacia koa A. Gray (koa) is the descendant of an ancient *Acacia* subgenus *Phyllodineae* (Fabaceae: Mimosoideae) ancestor that colonized the Hawaiian archipelago 5.1 to 6 million years ago (Le Roux et al. 2014). The descendants of this *Acacia* colonist evolved across a range of biomes throughout the Hawaiian Islands to become the present day "*Acacia koa* complex" consisting of: *Acacia koa*, *Acacia koaia*, and an intermediate amongst the two (Adamski et al. 2012). Koa inhabits elevations of 60 to 2300 m, and regions where 850 to 5000 mm of precipitation fall annually (Wagner et al. 1990). The distribution of koa is greatly determined by elevation and rainfall, and is distributed throughout Hawaiʻi, Maui, Lanaʻi, Molokaʻi, Oʻahu, and Kauaʻi where sufficient conditions exist.

Ecological importance of Acacia koa

The Hawaiian Islands contain some of the most remote terrestrial ecosystems on the planet. The Hawaiian hotspot has continuously produced a series of colossal shield volcanoes from the same location for at least 47 million years (Tarduno 2007). Historically, a relatively small number of successful plant colonizers arrived in Hawaiʻi circumstantially on the wind, atop waves, and by birds. These founders evolved to form the modern composition of native Hawaiian forests (Carlquist 1980). Koa is the largest canopy tree inhabiting Hawaiian forests. As atmospheric nitrogen fixing trees of the Fabaceae (Allen and Allen 1936), koa trees generate fertile soils through the addition of nitrogen from their decaying leaf litter (Idol et al. 2007; Scowcroft et al. 2004). This nutrient input forms a foundation for many ecosystems in Hawaiʻi. Thus, koa is considered a keystone species that many plants and animals depend upon (Welsbacher 2003).

Koa-dominated forests provide essential habitat for endemic plants; including 87 species that are considered endangered under the Endangered Species Act (ESA);

30% of all endangered plants in Hawai‘i (Baker et al. 2009). Many of these endemic plants grow only in the understory of koa-dominated forests, and are both an essential food source and habitat for endemic Hawaiian forest birds (Scott et al. 1986). Thousands of insects dwell on koa trees, including over 50 endemic species that evolved to feed only on koa (Wilkinson and Elevitch 2003). Prior to human settlement, Hawai‘i was home to at least 113 endemic bird species (VanderWerf 2012). Hawai‘i has experienced more avian extinctions than anywhere else in the United States (Scott et al. 1986). Since the arrival of the first humans in Hawai‘i, at least 71 species of endemic birds have gone extinct (Olson and James 1982; Scott et al. 2001; Wallace and Leonard 2011). Of the remaining 42 endemic bird species, 30 dwell in koa forests with 17 considered endangered under the ESA (U.S. Fish and Wildlife Service 2006). Losses of koa forest resulted in the extinction of koa finches (*Rhodacanthis*); which were dependent on koa seeds as a food source (James and Price 2007).

Cultural and economic importance of Acacia koa

Koa has been a vital resource in Hawaiian society for hundreds of years, and is revered for its alluring beauty, monumental stature, and unrivaled utility in shipbuilding. Koa forests were more widespread in the past, and held exceedingly large individuals, which today are mostly lost. These large koa trees were the foundation of Hawaiian seafaring culture, and were used to craft massive trans-Pacific voyaging canoes of over 100 feet in length (Wilkinson and Elevitch 2003). Hawaiian canoes are considered to be some of the most impressive ocean trekking vessels in the world (Holmes 1981), and would not have been possible to construct without access to the massive endemic koa trees of the Hawaiian Islands. Koa is Hawaiian for warrior (Kepler 1998). The brawny personification of this tree gives credence to the strength of its wood, enormous size potential, and ability to be crafted into massive vessels capable of navigating the most extensive ocean on the planet. Even today, the largest koa trees are referred to as “canoe trees.” Unfortunately, these large trees are exceedingly rare. In 1990, the Native Hawaiian Culture and Arts Program attempted to build a traditional Hawaiian doublehulled canoe out of koa, but was unable to find any trees large enough for construction (Wilkinson and Elevitch 2003). Koa was also used to make smaller canoes for fishing and racing, some of the original surf boards, paddles, bowls, weapons, and framing for huts (Holmes 1981; Wilkinson and Elevitch 2003). For centuries, the Hawaiian people have been cognizant of the holistic interconnectedness between montane watersheds, human populations, and reef ecosystems (Williams 1997). Prior to 1778, high-elevation forests had restricted access under the kapu system, with many koa trees being protected in those locations (Wilkinson and Elevitch 2003).

Koa forestry has been assessed to be potentially more profitable than cattle ranching (Goldstein et al. 2006). Koa wood is coveted for its vibrant beauty and is one of the most valuable hardwoods in the world. A variety of wood figure-types can occur, with the most highly regarded specimens being described as curly (Skolmen 1974). Wood color ranges from pale blonde to dark chocolate (Dudley 2007). Potter (2006) describes koa's stunning appearance, "Koa wood is generally known for its red to brown color and for its light-refractive characteristics, known as chatoyance. This attribute can lend a hologram-like effect to finished wood surfaces, adding to the visual allure of koa items." Highly desirable "instrument grade" koa can fetch $41,400 per thousand board feet (Scowcroft et al. 2010). Koa is utilized to produce a variety of specialty hardwood products including fine furniture, veneers, musical instruments and crafts (Dudley 2007).

Habitat loss and degradation of native Hawaiian forests

Compared to continental tropical forests, Hawaiian forest ecosystems contain a low diversity of plant species as a result of their oceanic isolation, and are exceptionally vulnerable to degradation from human land-alterations and introduced invasive species (Simon 1987; Stone et al. 1992). This vulnerability stems from Hawaiian species evolving largely in the absence of natural predators. As a result, many of these plants have lost their defenses such thorns and toxins, leaving them defenseless to an onslaught of invasive organisms (Olson 2004). Between 1100 and 1650 A.D., native lowland plant communities were lost and/or greatly altered due to agricultural development (Kirch 1985, 1994), and the spread of Polynesian rats (Cuddihy and Stone 1990). Pollen records provide evidence that koa once inhabited lowland forests prior to human settlement (Athens et al. 2002). Clearing native forests for intensive agriculture was extensive in upland locations above 750 m on the island of Hawaiʻi (Cuddihy and Stone 1990). Most notably were the Kona (Handy and Handy 1972) and Kohala (Kirch 1985) field systems. Large field systems were also present in Waimea (Menzies 1920), Ka'u (Newman 1972), and along the slope of the Hamakua coast (Cook 1967). These locations were once home to species-rich forests (Rock 1913; Rosendahl 1972; Kirch 1985).

Since European contact, anthropogenic impacts on the Hawaiian Islands intensified. The harvesting of koa began on an industrial scale in the 1830s (Jenkins 1983). Construction of road systems, where draft animals could skid large, previously inaccessible koa logs; felled by modern steel logging equipment, enabled the loss of much of the remaining koa forests (Cuddihy and Stone 1990). The whaling industry was the most dominant economic activity in Hawaiʻi from the 1820s to 1860s (Cuddihy and Stone 1990). In 1846, 736 ships arrived in Hawaiʻi to hunt whale. Decades of incessant whaling resulted in much of the forests surrounding ports being cleared to melt whale blubber into oil, this was said to have had an "appreciable effect in reducing forest areas" (Kuykendall 1938; Cuddihy and Stone 1990). After centuries of habitat loss, koa's distribution has predominantly been relegated to fragmented, high-elevation populations (Whitesell 1964). Wilkinson and Elevitch (2003) estimate that only 10% of the original koa forests remain.

Alien plants and animals are a primary cause of the numerous plant extinctions that have occurred in Hawaiʻi (Vitousek et al. 1987). A majority of the problematic species of plants and animals were brought after 1778 (Cuddihy and Stone 1990). Immense tracts of koa-dominated forests were cleared on the islands of Hawaiʻi and Maui to ranch introduced livestock and develop sugarcane plantations (Culliney 1988).

By the end of the 19th century, most of the upland koa forests on Mauna Kea were cleared for cattle ranching (Henke 1929; Tummons and Dawson 2002). Ranch and rangeland climaxed in 1960 with 52% of all land in the state put toward cattle production (Baker 1961). This included 65% of total land area on the island of Hawai‘i. Cattle were the first ungulates to be introduced to Hawai‘i by Captain Vancouver in 1793 (Tomich 1986). King Kamehameha placed kapu (hunting prohibition) on the new food source to increase their population size (Barrera and Kelly 1974). By the 1820s, much of the Mauna Kea landscape was infested by the exploding feral cattle population (Douglas 1914), with widespread damage to habitats recognized by the 1850s (Hillebrand 1856). Multiple destructive animals were introduced and became established in Hawai‘i including: feral goats, feral pigs, feral sheep, mouflon sheep, axis deer, and mule deer (Cuddihy and Stone 1990), which readily consume young koa seedlings and root sprouts (Baldwin and Fagerlund 1943; Scowcroft and Hobdy 1987; Spatz and Mueller-Dombois 1973).

At present, no less than 4,600 non-native plant species have been introduced to the Hawaiian Islands, with at least 800 having become naturalized (St. John 1973; Smith 1985; Motooka et al 2003). Of these naturalized plants, at least 86 are problematic, and 28 commonly invade intact native habitats (Smith 1985). Hundreds of alien grasses have been introduced to Hawai‘i (St. John 1973); including species that directly compete with koa such as: kikuyugrass (*Pennisetum clandestinum*), sweet vernalgrass (*Anthoxanthum odoratum*), common rush (*Juncus effuses*), common velvetgrass (*Holcus lanatus*), meadow rice grass (*Microlaena stipoides*), and molasses grass (*Melinis minutiflora)*. Many of these grasses were introduced in the 19th century to supply a continual forage source for immense herds of cattle (McClelland 1915; Hosaka 1958). Introduced pasture grasses have become one of the most limiting factors of koa regeneration. Competing vegetation (typically grasses) can exploit large quantities of soil-water and create droughty conditions for adjacent tree seedlings (Denslow et al. 2006; Holl 1998; Holl et al. 2000). Invasive grasses suppress native flora through the formation of expansive stolenous and rhizomnous mats. Once invasive grasslands become established, a grass-fire cycle typically ensues, making regeneration of fire intolerant native species exceedingly difficult (D'Antonio and Vitousek 1992). As a shade-intolerant species (Whitesell 1990), koa is vulnerable to regeneration failure and subsequent forest succession when alien plants overshadow their understory, and preclude regeneration.

Climate change: drought and soil moisture implications

Much of koa's range is located in areas that experience seasonal drought. Hawai'i has experienced a significant warming trend over the past century (Giambelluca et al. 2008), particularly at higher elevations (Diaz et al. 2011). Increasing temperatures have been coupled with a significant decrease in annual precipitation, particularly over the past 30 years (Chu and Chen 2005). Increasing dry-season solar radiation has been coupled with decreasing dry-season cloud cover and a reduction in the size of the mid-elevation cloud zone (Giambelluca et al. 2014), where much of the distribution of koa is located. Precipitation events have trended toward lighter precipitation events; away from frequent moderate to heavy rainfall events with a 13.5% decrease in total wet season precipitation (Chu et al. 2010; Elison Timm et al. 2011). Less precipitation has resulted in significant decreases in stream flow and base flow (Bassiouni and Oki 2012).

Climate model simulations robustly forecast a decrease of mean precipitation in mid-latitude and subarid regions, including Hawai'i (Christensen et al. 2007). A future decrease in mean precipitation will strongly burden soil moisture supplies. Climate change induced decreases in available soil moisture in conjunction with increases in solar radiation and temperature (Seneviratne et al. 2010), will greatly stress outplanted seedlings.

Transplanting and water stress challenge seedling survival

A planted seedling's capacity to promptly initiate new root growth, and colonize adjacent soils, largely determines establishment success (Sands 1984; Burdett 1987; Grossnickle 2005). Seedlings are exposed to stresses after lifting, transporting, and transplanting into new field environments. Limited soil moisture is a major contributor to seedling water stress and transplant shock (Haase and Rose 1993). Transplant shock is a result of outplanted seedlings not initially being connected into the hydrologic cycle of the field site (Grossnickle 2005). Seedling growth can be significantly reduced when planted into moisture-limited soils (Kaufmann 1977).

Water stress in seedlings must be minimized after planting in order to promote establishment, survival, and growth. After outplanting, however, plants can only uptake water via roots that are in direct contact with soil-water. Outplanted nursery stock have greatly confined root systems, which, at the time of planting, are limited in size to the volume of the growing container. When surrounding soils are dry, planted seedlings can rapidly become desiccated due to this root confinement, with only the periphery of the root plug being in direct contact with the soil interface. A negative feedback loop of establishment may become initiated. Plants combat fatal desiccation by closing their stomata, thereby halting the uptake of water. As a consequence of stomatal closure, atmospheric carbon dioxide is impeded from entering the stomata, and photosynthesis ceases. Photosynthetic discontinuance hinders plant growth and subsequent root system expansion to mitigate water stress. Burdett (1990) posits that water stress in recently outplanted seedlings is typical due to this limited root-soil junction. This is in contrast to naturally established seedlings, which can have (depending on soil conditions) root systems that are several meters in diameter, and downwardly trained toward sources of greater soil-moisture (Burdett et al. 1984).

Subsequent root growth after planting is imperative to mitigate water stress, and initiate the positive feedback model of seedling establishment (Burdett 1990). The positive feedback model of seedling establishment is a useful characterization of how outplanted seedlings become established into new environments (Burdett 1990). Pinto and others (2012) summarize this model as: water uptake increases stomatal conductance, which increases photosynthesis, which increases new root growth in deeper and moister soils, which allows for an even greater amount of water uptake. Because water stress is a foremost constraint in dryland outplantings, it is essential that root systems of nursery-grown seedlings embody characteristics that result in a prompt egress of roots to promote establishment and growth in the field.

Overcoming establishment constraints

A host of establishment constraints challenge koa reforestation, and must be overcome for outplanted stock to successfully grow into mature stands that support abounding native biological communities. Research over the past century has worked toward the identification of these restoration impediments. Thousands of acres of land, which were once intensively used for sugar cane production and cattle ranching, have been abandoned in recent years due to declining profitability (Scowcroft and Jeffrey 1999). This poses an opportunity to re-establish native forest species across these landscapes (Parrotta et al. 1997; Dudley 1997). There is at present, a great awareness to the importance of restoring the Hawaiian landscape, principally with endemic species such as koa, which provide essential ecosystem services.

Reforestation can be expensive, and typically involves multiple complex interactions between landowners, nurseries, and forest managers that can span extensive amounts of time. It is therefore essential that all necessary actions be executed toward a pre-defined, field objective in the future. The Target Plant Concept (TPC) is an established, objective-driven approach to establishing plants (Rose et al. 1990; Landis and Dumroese 2006). Identifying characteristics of seedlings that are quantitatively associated with reforestation success is the bedrock of this concept (Rose et al. 1990). Seedling quality can be an influential indicator of outplanting success

(Jacobs et al. 2004, 2005). Attributes of reforestation success are identified as targets of the TPC, and methods to attain those targets are delineated in a systematic process. For example, in Western Canada, seedling survival rates increased from 54% in 1982, to 87% in 1990 (Brown 1993). Hawkins (2011) attributes improvements to successful research extension and communication between scientists and nursery managers. Survival rates were greatly improved by: site preparation, careful handling of stock during planting, improved nursery cultural practices to increase seedling vigor, and adapting stocktype to the environment in which they are to be outplanted (Brown 1993). Seedling quality can only be determined in the field, in relation to pre-defined survival and growth objectives (Duryea 1985; Rose et al. 1990).

Summary

The koa tree embodies tremendous ecological, economic, and cultural value. The distribution of this iconic species has contracted since the settlement of Hawaiʻi from copious regeneration impediments. Multiple constraints challenge the large-scale reintroduction and field establishment of koa seedlings. It was recognized in the past that seedlings outplanted in their wild habitats, with low soil-moisture, and lacking irrigation, were more susceptible to drought-related mortality (Landis et al. 2010; Miller and Budy 1974). Upon outplanting, the relative shortage of soil-water in the field, compared to nursery irrigation, will typically result in transplant shock (Burdett 1990; Haase and Rose 1993). Climate change has the potential to severely exacerbate soil moisture limitations in some parts of Hawaiʻi and increase transplant shock. It is useful to investigate techniques that could promote hardiness in koa seedlings and advance their capacity to trounce field establishment limitations. Establishing nursery-grown stock in habitats that contain low soil-moisture, necessitates the employment of techniques throughout nursery culture that modify morphological and physiological attributes of that stock to ensure survival and adequate growth toward a definitive objective. Modifications to plants in nursery culture strongly influence the morphology and physiological condition of plants (Bayley and Kietzka 1997; Bigras and D'Aoust 1993).

Research Objectives

The overall aim of this thesis was to catalyze the positive feedback model of seedling establishment through nursery cultural techniques that modify koa seedling phenotype. Toward this aim, two techniques to modify koa seedling phenotype were investigated. The first technique was the implementation of a low-N hardening phase at the end of nursery culture. The objective of the low-N hardening phase experiments was to increase the root-to-shoot ratio of koa seedlings through a reduction in the amount of applied N in the final growth-phase in nursery culture. The use of distinct growth phases in nursery culture allowed for the precise supplementation of the quantities of mineral nutrients in order to satisfy nutritional requirements and meet growth objectives at each stage of seedling development. The utility of decreasing applied N during a hardening phase to increase seedling root-to-shoot ratio needs further investigation as a hardening tool for this species. The second technique used to modify seedling phenotype was the selection of differing container-types to produce contrasting root system architectures. This research expands on studies investigating the utility of large, deep, and air-pruning containers for producing native plants.

A pilot study preceding the experiments in this thesis was conducted in 2014 to measure the growth response of koa subjected to multiple levels of exponentially increased N applications. After seedling harvest, it was found that seedlings in this experiment had a notably low root-to-shoot ratio. It was speculated that applying N in an exponential manner could be antithetical to the common nursery objective of promoting root growth over shoot growth prior to seedling lifting by reducing the rate of N application. Further literature review following this pilot study resulted in the N hardening experiments found in this thesis, and focused on using distinct growth phases to study the effect of N deprivation during the final growth-phase in nursery culture.

In chapter two an experiment was conducted to investigate if reducing the amount of applied N during the final growth phase of nursery culture increases the root-to-shoot ratio of koa seedlings. Trubat and others (2008) found seedling biomass allocation of seedlings grown with a late phase N deprivation to vary between species. A dearth of research exists related to the growth response of koa seedlings subjected to a low-N hardening phase. It is a standard practice in nursery culture to reduce the amount of applied N in the hardening phase to promote stress-resistance mechanisms and reduce height and shoot growth (Mullin and Hallett 1983; Landis 1989; Trubat et al. 2008; Jacobs et al. 2014). Previous koa fertilization studies have investigated exponentially applied N (Diarra 2013) and the use of controlled-release fertilizer (Dumroese et al. 2009; Dumroese et al 2011). Pinto and others (2015) grew koa using fertigation and growth-phases with a low-N hardening phase, but did not explicitly study the effect of N deprivation during the final phase of nursery culture. Similar to the experiment conducted by Trubat and others (2008), the experiment in chapter two had a control treatment without a late phase N reduction, and a N hardened treatment where a low-N hardening phase was induced. This scenario raised the question of whether resulting seedling phenotypes are a product of the N hardening itself, or a difference in the total amount of applied N. To address this question, a third treatment with equal total applied N was executed without an N hardening phase.

Chapter three put the utility of N hardening seedlings to the test. Biomass allocation and field survival of seedlings grown with a late phase N deprivation has been shown to be species specific (Trubat et al. 2008). It would be useful to understand if the phenotype of koa seedlings produced from this N hardening technique conveys establishment and survival benefits in the field. Fertilization treatments in chapter three had an equal quantity of total applied N in each experiment. Chapter three also investigated the growth response of koa seedlings grown in two container-types (RootMaker® Express™ 18 and Deepot™ D25L containers). Experiments were designed with a factorial treatment structure (2 fertilization treatments × 2 container-types). The objective of the first experiment in chapter three was to examine leaf water potential of seedlings after transplant to gauge plant moisture stress in a simulated outplanting experiment. Measurements of plant moisture stress after planting provide a useful estimation of stocktype capacity to overcome moisture stress in the field, and become established. The second experiment in chapter three incorporated a dryland outplanting of these stocktype at a field site in the Koʻolau Mountains on the island of Oʻahu. The objective of the outplanting experiment was to examine whether N hardening koa seedlings conveys an enhanced survival effect in the field. A further aim investigated the contribution of container-type to survival and growth in the field.

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CHAPTER 2

REDUCING APPLIED NITROGEN IN THE FINAL GROWTH-PHASE OF NURSERY CULTURE DECREASES HEIGHT GROWTH, AND INCREASES THE ROOT-TO-SHOOT RATIO OF *ACACIA KOA*

Introduction

Attributes that influence a seedling's ability to rapidly establish in the field must be identified and quantified for successful reforestation or restoration of a given species (Rose et al. 1990; Duryea 1985). Survival and growth have been correlated with seedling root-collar diameter (RCD) and height (HT) at the time of planting (Thompson 1985; Jacobs et al. 2005). RCD is indicative of a plant's hydraulic transport capacity and correlates to root mass (Ritchie 1984, Mullin and Christl 1981). Thompson (1985) states, "A quality seedling should possess the largest diameter that confers an acceptable level of survival potential on that seedling for a given site". Seedling HT is indicative of, and highly correlated to leaf area which implicates a seedling's photosynthetic and transpiration capacity (Armson and Sadreika 1974). Excessive seedling HT and leaf area increase water uptake demand and can result in seedling mortality when planted on a site with insufficient soil-moisture (Cregg 1994; Jacobs et al. 2009). Outplanted seedlings that are exceedingly tall are also prone to wind rock, which can cause stem injury (Ritchie 1984).

Measuring seedling root quality and morphology in the nursery can provide insight into a seedling's potential to survive in the field (Rose et al. 1997; Jacobs et al. 2005). Certain measures have been shown to be more accurate predictors of establishment success in some hardwood species as opposed to others (Jacobs et al. 2005), and so it is useful to establish which measures are best for each species.

The root-to-shoot ratio (R:S) is the mass and/or volume of a seedling's root system relative to the mass and/or volume of a seedling's aboveground biomass (Lopushinsky and Beebe 1976). R:S is an influential quality assessor of outplanted seedlings as it indicates the capacity for water loss via shoots and leaves, and water uptake via roots at the time of planting (Lopushinsky and Beebe 1976; Racey et al. 1983; Ritchie 1984; Thompson 1985; Larsen et al. 1986). R:S is a standard measure of seedling morphology, which is commonly used to predict "drought avoidance potential," and establishment success (Grossnickle 2005, 2012). High-quality, outplanted seedlings have shoots that are not too large as to have a transpiration requirement that cannot be met by the roots at the time of planting (Cregg 1994).

Morphological and physiological attributes of seedlings can be modified through nursery cultural techniques, including tailored growth-schedules, to meet short and long-term objectives at the outplanting site (Bayley and Kietzka 1997). Fertilization in nursery culture can greatly alter seedling morphology and physiology (Landis 1985) and influence their ability to become established in the field (Bigg and Schalau 1990; Hawkins 2011). At least 14 elements have been identified as having an essential role in plant tissue structure and metabolism (Raven et al. 2005). It is important that nutrients be applied in a balanced proportion relative to each other, and at adequate concentrations (Salifu and Jacobs 2006). Optimally applied and balanced mineral nutrient concentrations can promote a plant's maximum growth in nursery culture (Hawkins 2011).

R:S can be manipulated through nutrient application in nursery culture. Nitrogen (N) is a crucial component in carbon compounds, it is a constituent of all amino acids and proteins, amides, nucleic acids, nucleotides, and polyamines (Epstein and Bloom 2005; Hawkins 2011). High levels of applied N result in greater shoot and leaf growth relative to root growth (Hawkins 2011). Hawkins (2007) found *Pseudotsuga menziesii* proportionally allocates more biomass to roots when a lower N concentration is applied in the nursery. This has also been shown in interior spruce (Miller and Hawkins 2003). The form of N applied also plays a significant function in the ensuing growth response. N applied as ammonium often results in excess shoot growth, whereas nitrate has been shown to preferentially promote lateral root branching over shoot growth (Everett et al. 2010; Hawkins 2011). During the final fertilization phase in nursery culture, reducing the amount of applied N to induce a mild nutrient stress can reduce shoot growth, and increase a seedling's R:S (Jacobs et al. 2014). This final reduction of N will hereon be referred to as N hardening and/or a low-N hardening phase.

Plant hormones (e.g., auxins and cytokinins) regulate growth allocation to roots and shoots respectively in relation to rhizosphere nutrient availability. Auxins are predominantly produced in shoot meristems and transported through the phloem to the root system, whereas cytokinins are produced in roots and are transported through the xylem to the shoots (Epstein and Bloom 2005). When plants are exposed to high N levels, shoot auxin concentrations diminish (Forde 2002), while root cytokinin concentrations increase (Takei et al. 2002). Increased cytokinin concentrations inhibit root expansion while stimulating shoot and leaf growth (Epstein and Bloom 2005). This is the mechanism being harnessed during an imposed nutritional hardening regime. When the availability of N decreases during the hardening phase, roots reduce the production of cytokinins (Takei et al. 2002). Plants respond to decreasing concentrations of cytokinins being received in shoots by producing auxins and, therefore, promoting root growth over shoot growth (Forde 2002; Epstein and Bloom 2005). Reducing applied N can be used to reduce height growth while simultaneously increasing root growth and R:S.

This study had two objectives. The first was to determine the contribution of a reduction of applied N in the final four weeks of nursery culture to the morphology and physiology of koa seedlings grown for twelve weeks. The second objective was to determine whether potential differences between N hardened and non-N hardened seedlings were due to the hardening regime itself, or the difference in total N applied between N hardened and non-N hardened treatments. It was predicted that a hardening regime would result in reduced shoot biomass, total height, and increased R:S.

Materials and Methods

Koa seeds were collected from a natural stand located at 1200 m elevation on the windward side of Hawaiʻi island. Seedlings were grown in an enclosed greenhouse at the Pope Laboratory of the University of Hawaiʻi at Mānoa in Honolulu, HI

(21°18'09.16"N, 157°48'54.42"W) using The RootMaker® Express™ 18 410 cm^{-3} containers held in RootMaker® Express 18 Injection-molded Trays (RootMaker® Products Company, LLC., Huntsville, AL). Containers were uniformly filled with a medium containing a 1:1 (v:v) mixture of sphagnum peat moss and vermiculite.

Nursery fertilization experiments with koa have shown that when controlled-release fertilizer (CRF) is applied for a 12 week growth regime; HT, RCD, and net photosynthetic assimilation is maximized between 2.3 (Dumroese et al. 2009) to 4.8 kg (Dumroese et al. 2011) total applied CRF per cubic meter volume of medium. These fertilization rates equate to 0.35 to 0.72 kg N m^{-3}. For this experiment, seedlings were also grown for 12 weeks. The target total N application of 0.432 kg N m^{-3} was chosen for the treatment without a low-N hardening phase, which is within the maximized application range reported by Dumroese and others (2009, 2011). While 0.315 kg N m^{-3} was the resulting target total N application after an imposed low-N hardening phase. This target total N quantity (0.315 kg N m^{-3}) was also used to for the treatment without a low-N hardening phase.

The experiment followed a completely randomized design (CRD) with a treatment structure of 3 fertilizer treatments and 5 replications arranged across 15 trays that were located on two greenhouse 3 m × 1 m benches. Three fertilizer treatments consisted of: 1. No reduction in N application after the rapid-growth phase (176 mg N $seedling^{-1}$), hereafter referred to as N176, 2. A reduction in N application following the rapid-growth phase (128 mg N $seedling^{-1}$), hereafter referred to as H128, and 3. No reduction in N application after the rapid-growth phase (128 mg N $seedling^{-1}$), hereafter referred to as N128 (Figure 2.1; Table 2.1). Randomization took place at the seedling level. Each seedling was randomly assigned a location within a tray using the Microsoft Excel list randomization feature. Trays within each treatment were the experimental units, and each held 9 seedlings, which were treated as subsamples. Tray location was randomized 3 times per week as to prevent potentially confounding microsite variability.

Nursery Culture

Seed coats were lightly scarified by hand using sand paper. Site of scarification was opposite the side of seed attachment to legume. Great care was given to prevent abrasion to the radical and endosperm. Seed imbibition is an essential stage in successful koa germination. Seeds were allowed to imbibe by submersion in deionized water for 24 hours. After imbibition on 28 April 2015 two seeds were sown per container at a depth 2 the breadth of the seed, into 200 total containers. During the germination phase, containers were lightly irrigated twice per day using a mist nozzle on a standard garden hose. Germination was considered complete after 14 days, at which time each container was thinned so that only one seedling populated each container. Containers in which no seeds germinated were removed. 135 of the remaining seedlings were randomly selected and randomly assigned a location within a tray using the Microsoft Excel list randomization feature.

Tray irrigation requirement was ascertained by means of the “scientist technique” (Dumroese et al. 2015) of the block-weight method (White and Mastalerz 1966). Irrigation timing was based on gravimetric container weights for each treatment, 65% (±5%) during establishment, rapid-growth, and hardening phases (Figure 2.2). Nutrients were supplied to individual seedlings as a custom-mixed fertigation solution. Seedlings were fertilized 3 times per week at a rate in accordance to each treatment’s respective fertilizer schedule (Figure 2.1; Table 2.1, 2.2). Concentrated stock solutions were prepared to create custom fertilizer solutions for individual trays. Three stock solutions were prepared, and consisted of: 1. Calcium Nitrate, 2. Monopotassium Phosphate/Magnesium Sulfate, and 3. General Hydroponics® FloraMicro Hardwater (General Hydroponics-USA, Santa Rosa, CA). Total N targets by treatment were: 1. 176 mg N seedling^{-1} (N176), 2. 128 mg N seedling^{-1}, (H128), and 3. 128 mg N seedling^{-1} (N128) (Figure 2.1). Fertilizer solutions were kept at a pH of 6.0 by additions of phosphoric acid or General Hydroponics® pH Up. To apply nutrients, a 40 mL aliquot of fertilizer solution was precisely and evenly applied by hand, to each seedling. This volume was calculated to bring containers back to field capacity from a 65% dry down. Between fertilizer events, seedlings were hand-irrigated with water to field capacity as necessary.

Sampling

The morphological plant growth characteristics of height (HT) and root-collar diameter (RCD) were measured weekly through nursery culture. Seedling HT was measured from the medium surface to the apical meristem. RCD measurements were taken at the root-collar. HT, RCD, root dry mass (RDM), leaf dry mass (LDM), stem dry mass (SDM), and leaf N concentration (LN%) were measured on all seedlings post-harvest on 29 July 2015. Destructive sampling was conducted on all seedlings to determine RDM, LDM, SDM, and LN%. First, root systems were carefully washed free of all media. Second, seedlings were severed at the root-collar and roots, leaves, and stems were separated and dried at 60°C for 72 hours. Shoot dry mass (SHDM) was calculated by summing LDM and SDM. Following drying, RDM, LDM, and SDM were measured and subsequently used to assess seedling root-to-shoot ratio (R:S). Composite samples of leaf tissue were produced to analyze LN%, and were measured by: Agricultural Diagnostic Service Center, University of Hawaiʻi at Mānoa. Leaf N content (LNC) was calculated by multiplying LN% by LDM.

Statistical Analysis

To analyze plant growth characteristics, means from tray replicates for each treatment were analyzed using a one-way ANOVA to evaluate treatment effects ($P<0.05$) for each response variable (HT, RCD, RDM, LDM, SDM, SHDM, R:S, LN%, LNC) after nursery culture and destructive harvest. For each response variable, normal quantile plots were constructed for each treatment and the pooled residuals from all treatments to assess normality. A Brown-Forsythe test was ran for each response variable to determine the equal variance assumption for an ANOVA. The data met all assumptions for normality and equal variance, and thus no transformations were necessary. Treatment comparisons were evaluated using the Tukey HSD test; differences were deemed significant at α=0.05. Analyses were performed using SAS JMP Pro Statistical Software (Version 12.0).

Results

Treatment effects conferred differential responses for morphological measures (Table 2.3, 2.4; Figure 2.3, 2.4, 2.5, 2.6, 2.7). The H128 treatment with a low-N hardening growth-phase exhibited a 47 and 37% increase in R:S of seedlings compared to N176 and N128, respectively (p < 0.0001) (Figure 2.3). There was no significant difference in R:S between N176 and N128 (p = 0.4824).

During the rapid-growth phase, treatments that received 6.0 mg N application event^{-1} (H128 and N176) constructed aboveground tissues at an 11% greater rate relative to N128 which received 4.2 mg (RCD at 7 weeks; p < 0.0001) (Table 2.4; Figure 2.6, 2.7). The imposed low-N hardening phase in H128 reduced the rate of HT growth (Figure 2.6). Both H128 and N128 had a significantly decreased HT compared to N176 after 12 weeks (-18% and -11%; p < 0.0241). There was no significant difference in RCD between H128 and N176 (p = 0.1384). N128 had a 9 and 12% reduction in RCD compared to H128 and N176 (p < 0.0029). Final H128 RCD was not impeded as drastically as HT compared to N176 (-4.2% vs. -22.2%). H128 was also 9% shorter than N128, while having a 10% greater RCD.

Fertilizer treatments also conferred differences in biomass among seedlings (Table 2.3; Figure 2.3). Decreasing N fertilization after the rapid-growth phase (H128) significantly increased RDM by 22 and 56% compared to maintaining N fertilization rates at a continuous rapid-growth phase rate regardless of N (N176 and N128, respectively; p < 0.0017). Both H128 and N128 had a significantly decreased SHDM

17% and 27%, respectively, compared to N176 ($p < 0.0003$). N128 was 12% smaller in SHDM compared to H128 ($p = 0.0148$).

Both H128 and N128 had a significantly decreased SDM 20 and 30%, respectively, compared to N176 ($p < 0.0018$). N176 had a significantly increased LDM compared to H128 and N128 (17% and 33%; $p < 0.0007$). H128 had a significantly increased LDM compared to N128 (14%; $p = 0.0096$).

Decreasing N fertilization after the rapid-growth phase (H128) significantly decreased LN% compared to maintaining N fertilization rates at a continuous rapid-growth phase rate regardless of N (N176 (-21%) and N128 (-18%); $p < 0.001$) (Figure 2.4). Although, treatments that received an equal amount of total applied N (H128 and N128) did not have a significantly different LNC ($p = 0.4040$) (Figure 2.5). There was no significant difference in LN% between treatments that did not receive a low-N hardening phase, N176 and N128 ($p = 0.6720$). N176 had a significantly increased LNC compared to H128 and N128 (+48% and +37%; $p < 0.0001$).

Discussion

This study tested the hypothesis that reducing the amount of applied N in the final four weeks of nursery culture would increase the R:S of koa. The mechanism for koa to increase R:S was hypothesized to be the plant shifting its allocation of above- and below-ground biomass in response to a limited supply of N. This is a well-documented growth response in plants (Epstein and Bloom 2005; Hawkins 2011). Exploiting this physiological response was hypothesized to be a useful way to modify the seedling morphology of koa into an architecture more suitable for subsequent outplanting into sites where soil moisture is a limiting factor for seedling establishment.

A strength of this study was the investigation of potential differences between reduced N and continuous N, and if differences were due to the application timing itself (e.g. reduced after the rapid-growth phase), or the difference in total N applied regardless of growth phase. Trubat and others (2008) investigated the effects of a low-N hardening phase on Mediterranean species and found conflicting results regarding above- and below-ground biomass allocation, but did not account for the difference in total applied N. Differential results such as these give credence to investigating N hardening koa to assess if field survival benefits could be accrued. The results of this experiment show that the morphological attributes of N-hardened koa seedlings (grown for 12 weeks with 0.315 kg N m^{-3}) were not merely a result of having a different total N applied.

Treatment N176 was devised to demonstrate how H128 would have grown if the N application rate during the rapid-growth phase wasn't terminated for the subsequent low-N hardening phase (similar to Trubat et al. 2008). As expected, the increased total applied N from not having a low-N hardening phase, increased all above-ground morphological response variables, with the notable and expected exception of RM and R:S. This finding is consistent with the notion of plants increasing the growth of above-ground structures relative to belowground in response to increased N fertilization (Epstein and Bloom 2005; Hawkins 2011).

Planning a target seedling HT is important. Seedling HT is indicative of, and highly correlated to leaf area (Armson and Sadreika 1974). The leaf area present in excessively tall seedlings burdens the water uptake ability of root systems (Cregg 1994). Planting a seedling that is too tall often results in stem damage from wind rock (Ritchie 1984). This study demonstrates that a low-N hardening phase can be an effective tool to curtail HT growth of koa in nursery culture and could be used as a tool to achieve a target seedling HT.

It is notable that in H128, RCD growth was not impeded as drastically as HT compared to N176. H128 grew to have advantageous morphological attributes compared to seedlings with the same total N applied (N128). H128 had a decreased final HT, while having a greater RCD compared to N128. Having a seedling with a decreased HT and an increased RCD can confer increased survival and growth after planting (Thompson 1985; Jacobs et al. 2005). This study provides evidence that optimal seedling growth can be accrued using less N through the use of 3 custom growth phases (establishment, rapid-growth, and hardening) as opposed to 2 (establishment and rapid-growth).

The low-N hardening phase had a strong effect on leaf N concentration. There is a strong positive correlation between leaf N concentration and photosynthetic capacity (Field and Mooney 1986; Reich et al. 1997; Reich et al. 1999). A reduced leaf N concentration could implicate the seedling's ability to initiate the positive feedback model of establishment through this probable reduced photosynthetic capacity. Conversely, a reduced photosynthetic capacity may also aid establishment in moisture-limited soils as photosynthetic capacity is correlated to stomatal conductance and water demand (Chapin et al. 2002). The LN% in this study (2.3-2.9%) were similar to the shoot N concentrations Dumroese and others (2009) reported for 12 week-old, nursery-grown koa (2.2-2.78%). Dumroese and others (2009) found shoot N was maximized (3.85%) at 1.79 kg N m^{-3}. 3.85% is likely approaching koa's maximum shoot N concentration as next lower fertilizer treatment (1.43 kg N m^{-3}) was only 0.09% lower (3.74%). Further research would be useful to determine koa leaf N concentration following a low-N hardening phase with a higher total applied N. Interestingly, the considerable differences in shoot morphology between H128 and N128 were not present in total leaf N content (Figure 2.7) with these treatments having no significant difference in LNC.

The increased growth of H128 seedlings during the rapid-growth phase compared to N128, created a larger relative seedling size which persisted through the hardening phase. It is likely that N128 seedlings grew through some of the rapid-growth phase with a suboptimal dosage of applied N (4.2 mg N $application^{-1}$). N128 demonstrated a reduced growth-rate compared to H128 and N176 treatments that received (6.0 mg N application^{-1}) during their rapid growth phase (Figure 2.3, 2.4). A limitation of this study was not having an H176 treatment (equal total N as N176 but with a low-N hardening phase). For experimental purposes in the future, N application rates during the rapid-growth phase could be greater than (4.2 mg N application^{-1}), and potentially beneficial above (6.0 mg N application^{-1}) for koa grown in 410 cm^{-3} containers for 12 weeks. An increased amount of applied N (>4.2 mg N application^{-1}) and a resultant optimized growth-rate in the rapid-growth phase of treatments with continuous N application without a low-N hardening phase, could lead to different morphological growth responses between treatments.

Further study is needed to optimize nutrient application and multi-element proportion targets for koa in each growth phase. Salifu and Jacobs (2006) studied multiple rates of 15N-5P_2O_5-15K_2O fertilizer applied as a liquid solution to *Quercus rubra* seedlings in nursery culture to quantify fertility targets for that species. This approach would be a useful basis from which to optimize koa growth in the nursery, and then compare optimized target quantities with a low-N hardening phase (with an equal total N).

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Tables

Table 2.1. Nutrient application quantities (mg) per seedling per application event by treatment and growth-phase.

	H128			N128		N176	
Nutrient	**Establishment**	**Rapid-Growth**	**Hardening**	**Establishment**	**Rapid-Growth**	**Establishment**	**Rapid-Growth**
N	2.0	6.0	2.0	2.0	4.222	2.0	6.0
P	0.64	2.84	2.84	0.64	2.84	0.64	2.84
K	1.2	4.0	4.0	1.2	4.0	1.2	4.0
Ca	0.4	5.32	0.4	0.4	2.6	0.4	5.32
Mg	0.64	1.8	1.8	0.64	1.8	0.64	1.8
S	0.83	2.34	2.34	0.83	2.34	0.83	2.34
Fe	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Mn	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Zn	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Cu	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Mo	0.00032	0.00032	0.00032	0.00032	0.00032	0.00032	0.00032
Co	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002

Table 2.2. Fertilization event dates by treatment and growth-phase May through July, 2015.

Establishment Phase: H128, N128, N176

5/11	5/13	5/15	5/18	5/20	5/22	5/25

Rapid-Growth Phase: H128, N128, N176

5/27	5/29	6/1	6/3	6/5	6/8	6/10	6/12	6/15	6/17	6/19	6/22	6/24	6/26	6/29

Hardening Phase: H128 / Rapid-Growth Phase: N128 and N176

7/1	7/3	7/6	7/8	7/10	7/13	7/15	7/17	7/20	7/22	7/24	7/27

Table 2.3. Morphological characteristics of *Acacia koa* seedlings following 12 weeks of nursery culture (n=15). Treatments are: H128-reduction in N application following rapid-growth phase (128 mg N seedling^{-1}); N128-no reduction in N application following rapid-growth phase (128 mg N seedling^{-1}); and N176-no reduction in N application following rapid-growth phase (176 mg N seedling^{-1}).

Treatment	Height (cm)		Root-Collar Diameter (mm)		Root Dry Mass (g)		Stem Dry Mass (g)		Leaf Dry Mass (g)		Shoot Dry Mass (g)	
H128	44.11(1.54) *	a†	5.98(0.10)	a	2.50(0.09)	a	1.54(0.09)	a	2.73(0.07)	a	4.27(0.14)	a
N128	48.01(1.62)	a	5.46(0.04)	b	1.60(0.03)	b	1.36(0.04)	b	2.40(0.06)	b	3.75(0.08)	b
N176	53.90(0.71)	b	6.23(0.10)	a	2.05(0.07)	c	1.93(0.05)	c	3.19(0.06)	c	5.12(0.10)	c

*Statistical means and associated standard errors.
†Different letters within the same column indicate significant differences (α=0.05).

Table 2.4. Morphological characteristics of *Acacia koa* seedlings measured at the end of the rapid-growth phase (7 weeks; n=15). Treatments are: H128-reduction in N application following rapid-growth phase (128 mg N seedling^{-1}); N128-no reduction in N application following rapid-growth phase (128 mg N seedling^{-1}); and N176-no reduction in N application following rapid-growth phase (176 mg N seedling^{-1}).

Treatment	HT (cm)		RCD (mm)	
H128	32.00(1.19) *	a	3.90(0.06)	a†
N128	30.62(0.87)	a	3.52(0.05)	b
N176	32.16(0.62)	a	3.86(0.04)	a

*Statistical means and associated standard errors.
†Different letters within the same column indicate significant differences (α=0.05).

Figures

Figure 2.1. Schematic of fertilizer treatment schedules by macronutrient (N,P,K).

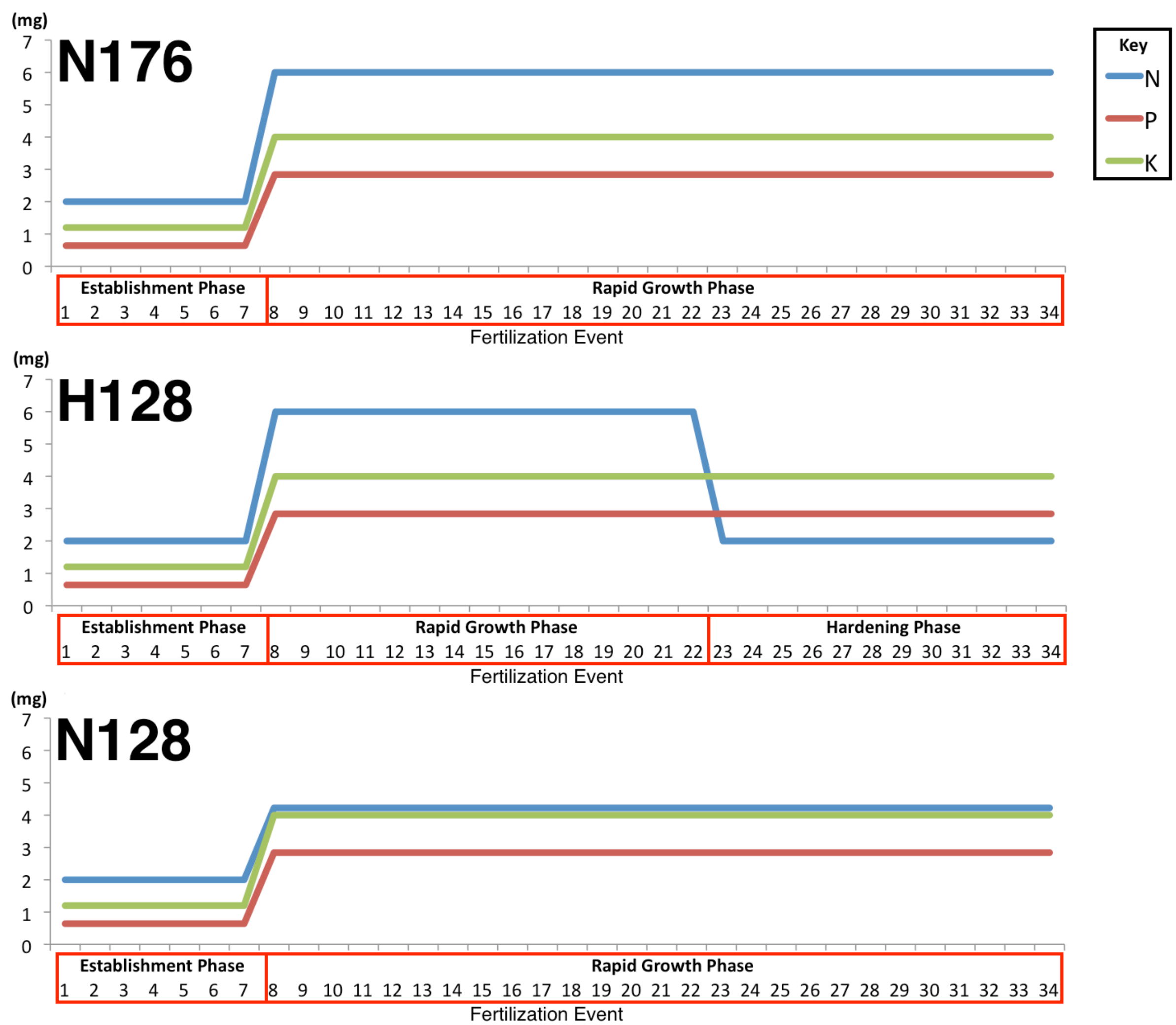


Figure 2.2. Schematic of fertilization and irrigation timing based of gravimetric water content.

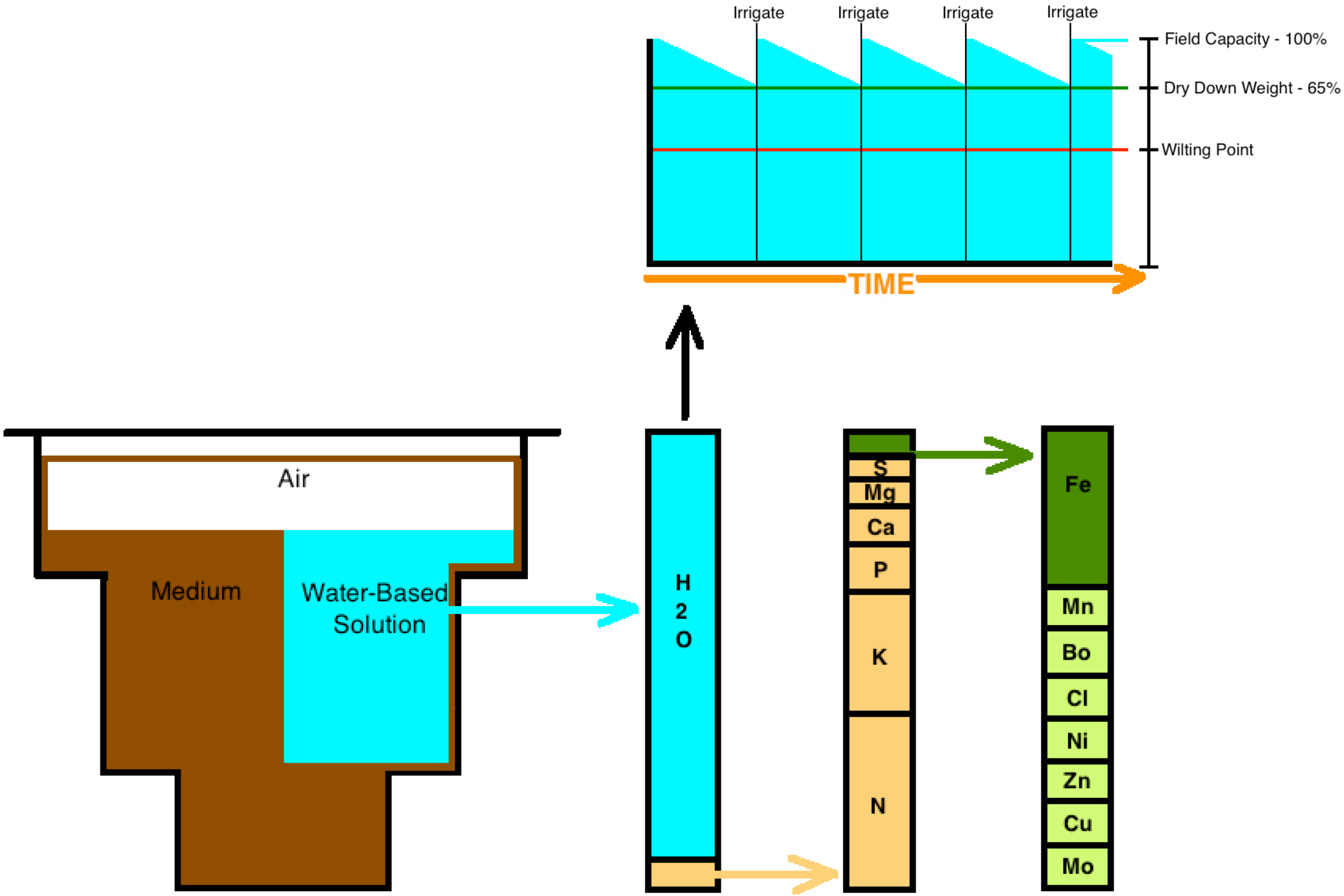


Figure 2.3. Mean (±SE) root-to-shoot ratio by treatment. Different letters indicate significant differences (α=0.05).

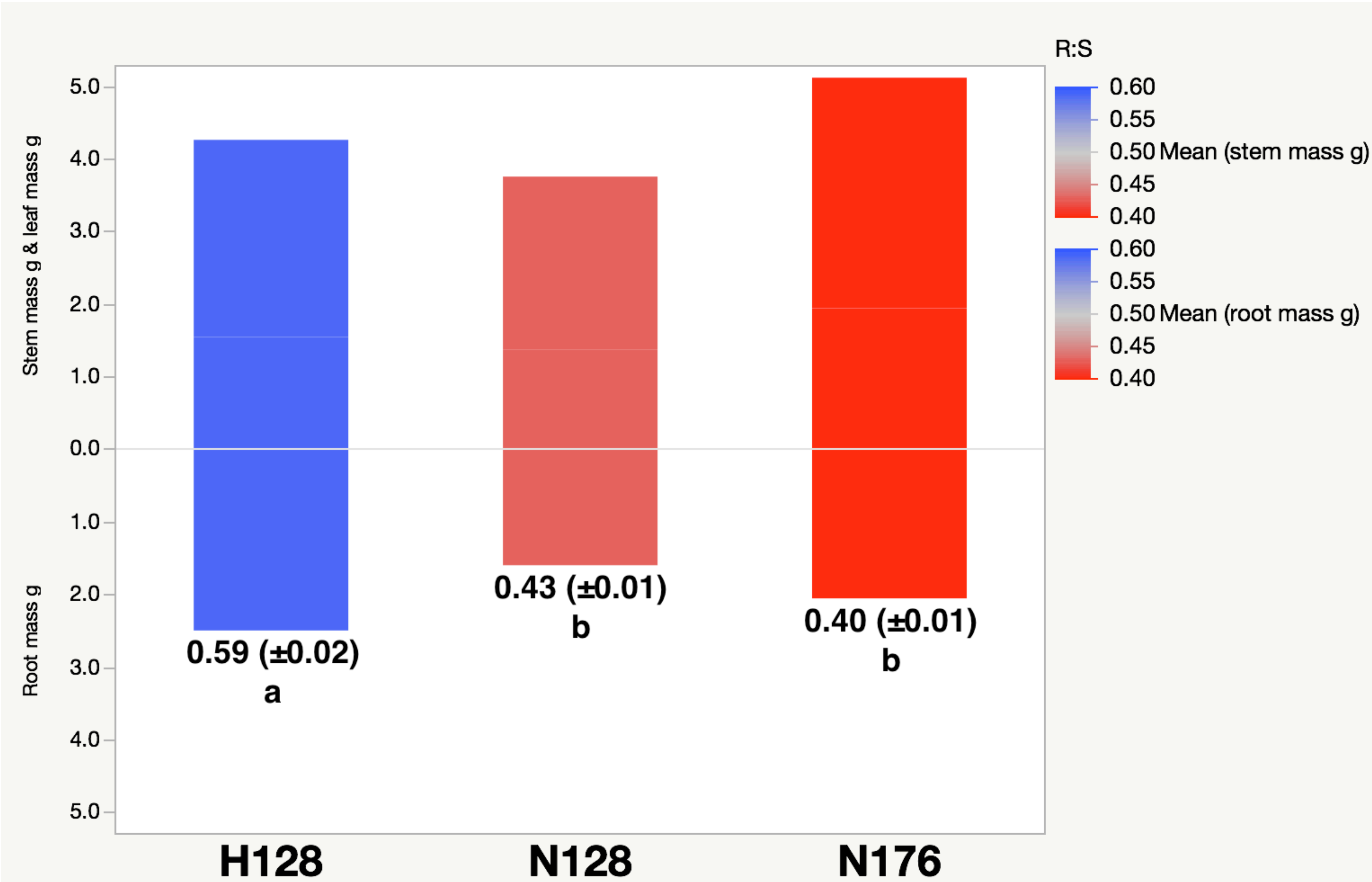


Figure 2.4. Mean (±SE) leaf nitrogen concentration (%) by treatment. Different letters indicate significant differences (α=0.05).

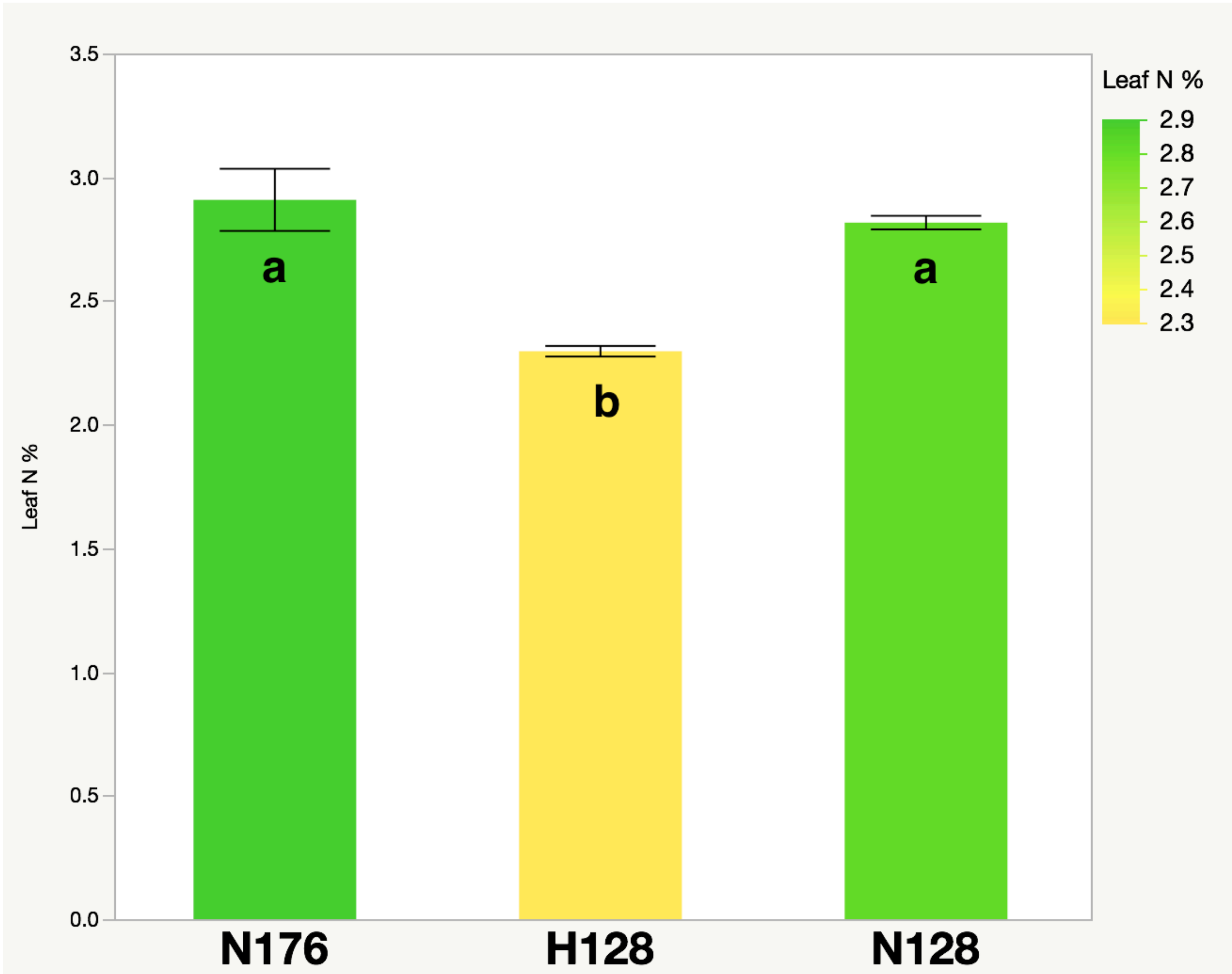


Figure 2.5. Mean (±SE) leaf nitrogen content (g) by treatment. Different letters indicate significant differences (α=0.05).

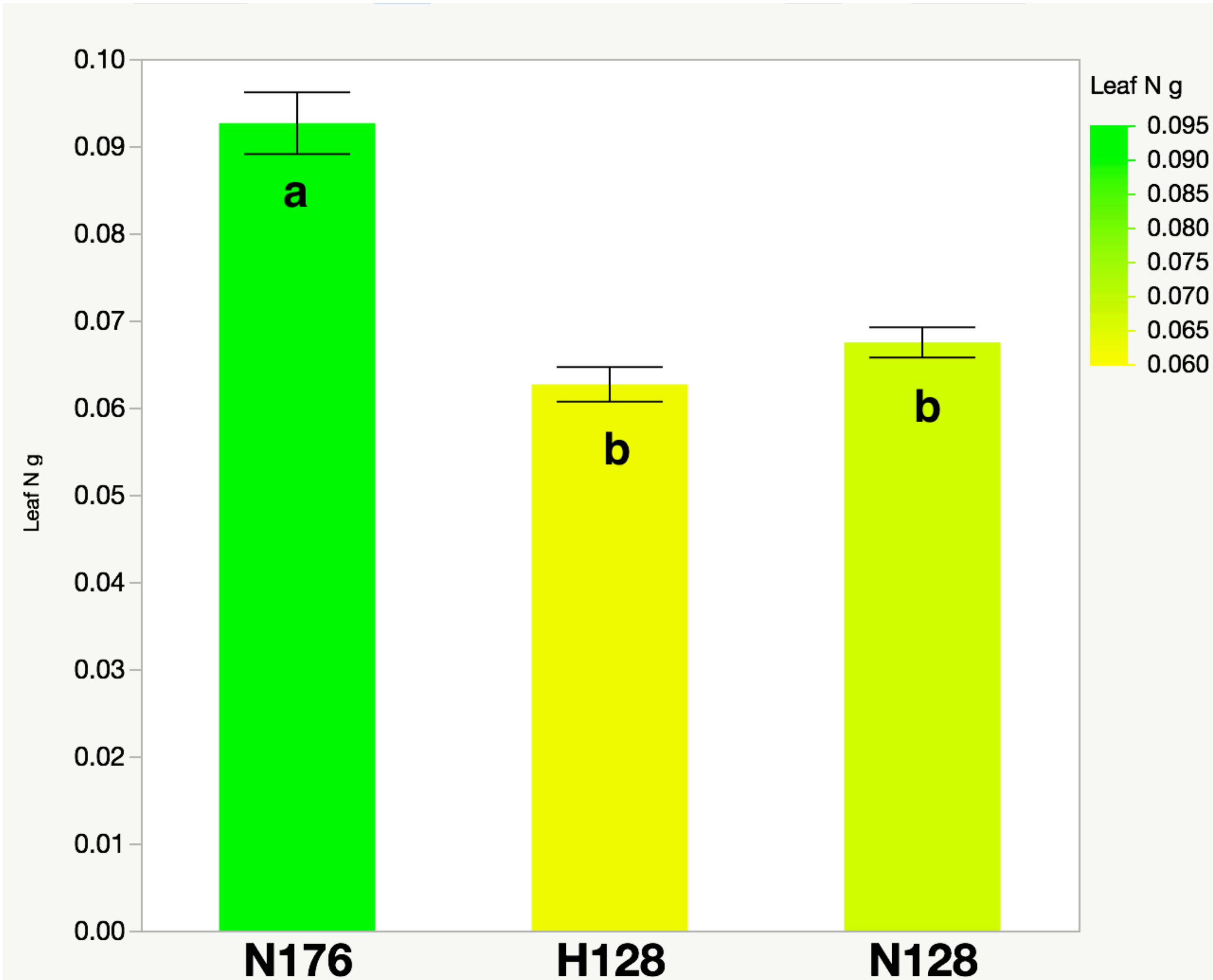


Figure 2.6. Mean seedling height (cm) by treatment through time. Low-N hardening phase initiation is depicted.

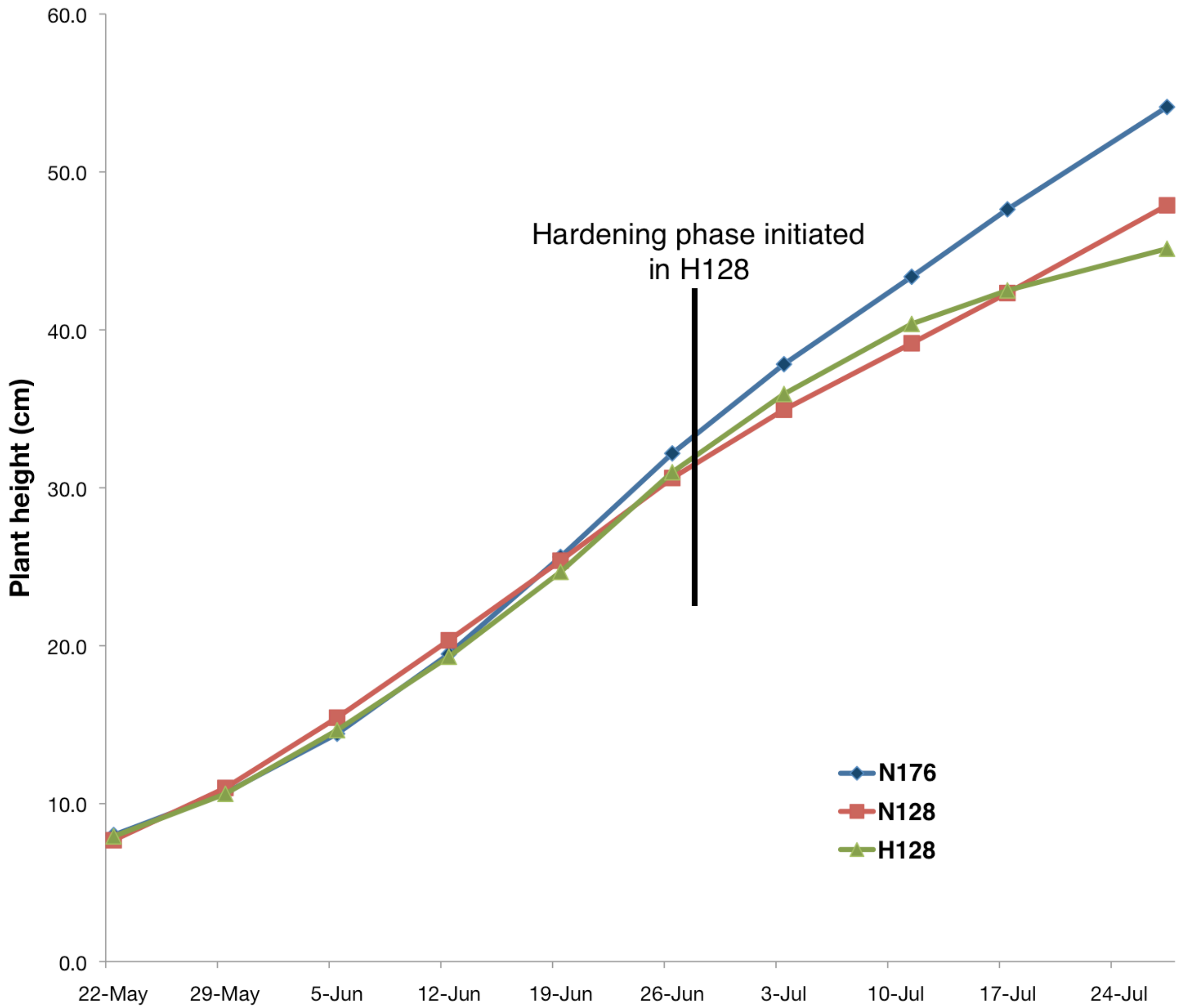
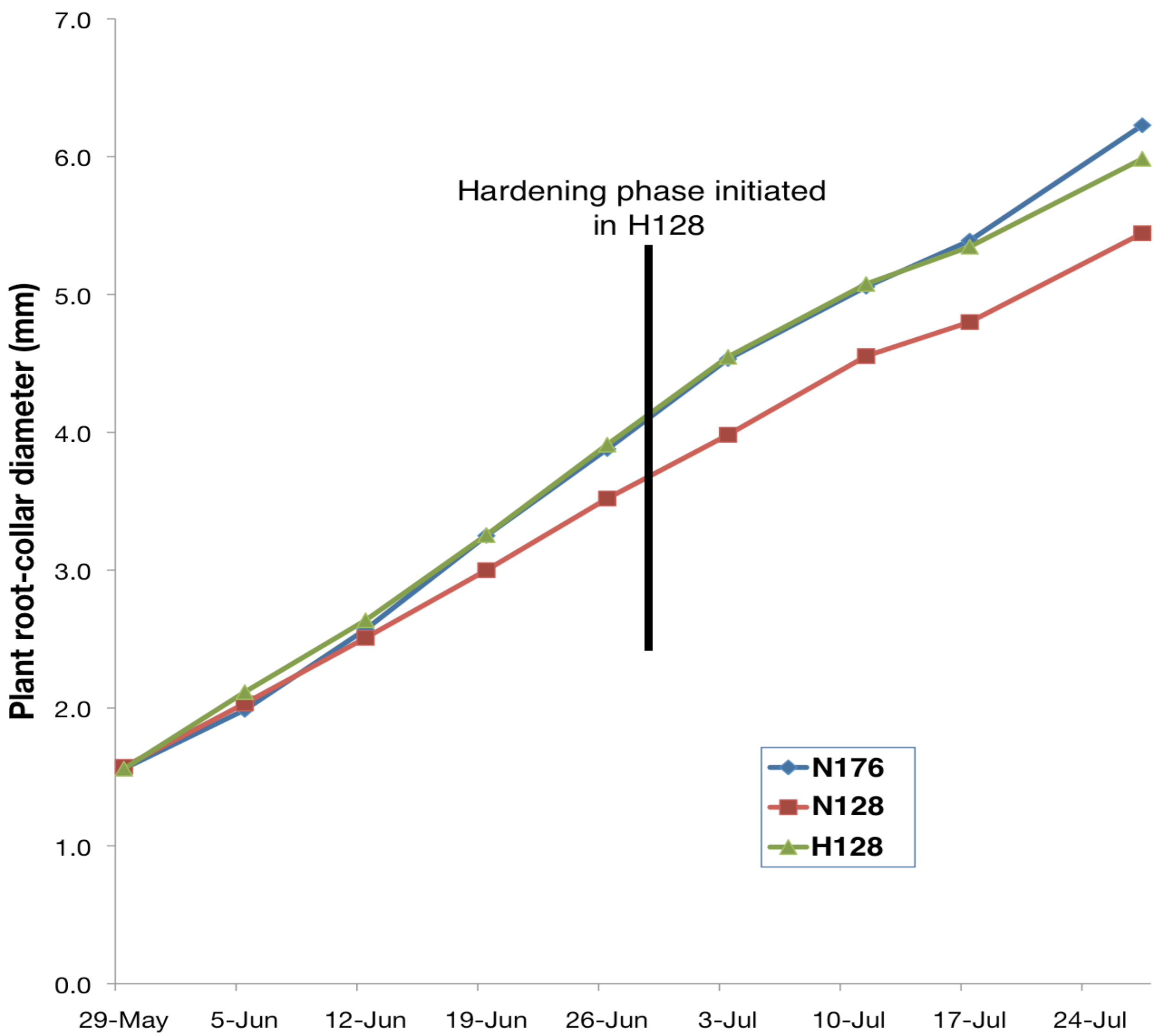


Figure 2.7. Mean seedling root-collar diameter (mm) by treatment through time. Low-N hardening phase initiation is depicted.



Images

Image 2.1. *Acacia koa* seedlings after 10 weeks of growth (12 July 2015).

Image 2.2. Root system of Nitrogen-hardened seedlings (treatment H128) at harvest (12 weeks of growth; 29 July 2015).

CHAPTER 3

EFFECTS OF CONTAINER-TYPE AND DIFFERING NITROGEN FERTILIZER REGIMES ON THE SURVIVAL AND GROWTH OF *ACACIA KOA* IN THE FIELD

Introduction

Many question the time and capital related investments required for reforestation with seedlings. Using container-grown seedlings, however, has been found to be a very effective method of reforestation compared to natural regeneration or other methods (McDonald et al. 2009). Container seedling stock can improve survival and growth for some species and has also shown to be effective on challenging sites such as those that are moisture-limited in dryland reforestation scenarios (McDonald 1991; Chirino et al. 2008). Selecting an appropriate stocktype for any given outplanting endeavor is a primary consideration in the planning process of reforestation. Limiting factors at the outplanting site are a primary drivers for this selection, but other factors such as production limitations, planting costs, and other logistics can influence this choice. In the end, the physical and physiological attributes such as size, structure, age, and nutrient status of the nursery produced seedlings (referred to as stocktype) are a holistic choice with many inputs.

The benefit of growing seedlings in nursery culture is the ability to control the growing environment. Nursery managers can craft a specific and optimized target seedling morphology through container selection, nutrition, and manipulation of the growing environment. Seedling morphology can be manipulated through container selection to overcome various field limitations. Root system architecture can be modified through container selection, and differs greatly between container designs (Landis et al. 1990; Aphalo and Rikala 2003; Davis and Jacobs 2005). Abiotic and biotic factors influence root system development, and are species specific (Cairns et al. 1997). It should be the primary objective of nursery managers to produce stocktype with characteristics that promote establishment under harsh field conditions.

It is crucial that seedlings are grown to an appropriate size for any given species and container-type. Container stock grown for an excessive amount of time can become root-bound (Dickerson 1974); this condition influences an initial root architecture that persists through the life of the plant. Roots of root-bound systems constrict each other, as they grow larger and thicker through time (South and Mitchell 2006). Poorly structured root systems can predispose a tree to becoming structurally unstable, and vulnerable to wind blow (Lindgren and Orlander 1978).

Seedlings grown in larger or longer containers have a proclivity to survive in conditions with low soil-moisture (Pinto et al. 2011b; Pinto et al. 2015; Villar-Salvador et al. 2012). Larger containers produce seedlings with more roots, a greater root-collar diameter, and increased height. Larger stocktypes have a greater capability to compete for resources with vegetation already present at the outplanting site (Overton and Ching 1978; Newton et al. 1993; Thiffault et al. 2003; Jobidon et al. 2003). Larger outplanted stocktypes typically sustain a larger size and growth-rate over time when compared to smaller outplanted stocktypes (Jacobs et al. 2005). Larger seedlings can be grown by increasing: container size, applied fertilization, and irrigation (Endean and Carlson 1975; Scarratt 1972; Timmer and Miller 1991).

A planted seedling's capacity to promptly initiate new root growth, and colonize adjacent soils, largely determines establishment success (Sands 1984; Burdett 1987; Grossnickle 2005). New roots are often classified into two categories: 1) fibrous roots that are primarily associated with water and nutrient absorption and are typically short feeder roots and, 2) pioneer roots that are long and have the primary purpose of seeking out soil water (Lyford and Wilson 1964; Wilcox 1964; Horsley and Wilson 1971; Kolesnikov 1971; Lyford 1980; Sutton and Tinus 1983). Fibrous root systems are typically short-lived, and are primarily constructed for the purpose of water and nutrient absorption, and do not typically undergo secondary growth (Polverigiani et al. 2011). Pioneer roots typically have a fast rate of growth, and are primarily constructed to explore vertical and horizontal soil stratums for sources of soil moisture and nutrients, and typically have long average life expectancies as they undergo secondary growth (Polverigiani et al. 2011; Wells and Eissenstat 2003; Zadworny and Eissenstat 2011).

Deep containers and root-pruning containers confer structural benefits to seedling root systems in preparation for outplanting. Chirino and others (2008) summarizes the benefits of deeper containers as, "Container depth can determine root system growth and tap root length, and thus it can modify soil colonization in deep soil horizons. Growth of new roots out of the root plug for deep soil colonization is a critical factor for seedling survival". On sites with low soil-moisture, seedlings that have larger and deeper root systems exhibit an inclination toward increased survival and growth (Amidon et al. 1982; Rose et al. 1997; Chirino et al. 2008). Facilitating taproot growth with deep containers gives outplanted seedlings an advantage in accessing deep soil moisture (Canadell et al. 1996).

Root- and air-pruning containers can create a more fibrous root system (Larson 1975). Crafting a seedling with increased root fibrosity can aid establishment due to the large quantity of active root tips that will subsequently grow into the surrounding soil after planting (Thompson 1985; Davis and Jacobs 2005). Root-training and air-pruning containers create extremely fibrous root systems with profuse lateral branching and prevent roots from spiraling. This container-type promotes lateral root training and assists the horizontal spread of root systems post-planting (Burdett 1981).

Moisture stress in seedlings must be minimized after planting in order to promote establishment, survival, and growth. Limited soil moisture is a major contributor to seedling water stress and transplant shock (Haase and Rose 1993). Transplant shock is a result of outplanted seedlings not initially being connected into the hydrologic cycle of the field site (Grossnickle 2005). Seedling growth can be significantly reduced when planted into moisture-limited soils (Kaufmann 1977). Plant moisture status and stress can be determined through the use of a pressure chamber (Scholander et al. 1965). In this method, pressure is increased around a leaf's petiole until xylem sap appears at the cut end of the petiole at which time a measure of leaf water potential (LWP) is taken (measured in MPa Ψ). LWP is a useful indication of plant water status and moisture stress. High plant moisture stress levels cause can reduce or stop photosynthesis

(Scholander et al. 1965). Plant moisture stress measurements can be used to assess a plant's water requirement and how well the plant is adapted to its environment (Haase and Rose 1993). Measuring plant moisture stress gives an indication of a plants ability to grow and can be used as a guide for improving stocktypes to survive in adverse conditions.

Existing research concerning the restoration of Hawaiian forest ecosystems with nursery-grown *Acacia koa* forms a constructive foundation to build upon. Much of this research has endeavored to overcome extensive constraints at Hawaiian outplanting sites from invasive plant competition. It is established that the removal of ungulates from Hawaiian outplanting sites is a prerequisite for seedling survival, and that this can be accomplished through the construction of fenced-off enclosures (Baldwin and Fagerlund 1943; Scowcroft and Hobdy 1987; Spatz and Mueller-Dombois 1973). Koa demonstrates increased survival and growth when competition from invasive grasses is ameliorated by means of site pre-treatment with herbicide (Scowcroft and Adee 1991; Pinto et al. 2015). Pre-treating a site with herbicide to suppress grass competition can result in greater volumetric water content for a year after planting (Pinto et al. 2015). Initial grass suppression accelerates outplanted koa stock toward canopy closure (Pinto et al. 2015). Previous field experiments have demonstrated lower koa survivorship when outplanted on sites that have not been pre-treated with herbicide to suppress grass competition (Scowcroft and Adee 1991; Davis et al. 2011).

Optimal container-type and shape varies between species (Endean and Carlson 1975; Carlson and Endean 1976). Many koa reforestation projects have utilized relatively small stocktype (Scowcroft and Adee 1991; Whitesell 1990) More recently, koa grown in large containers have been shown to confer increased survival and growth (Dumroese et al. 2011; Pinto et al. 2015). The proximity in which containers grow to each other effects height and root growth, with a lower density promoting increased root and root-collar diameter growth, and reduced height growth (Aphalo and Rikala 2003; Dominguez-Lerena et al. 2006; Pinto et al. 2011a, b; Aghai et al. 2014). Testing two container-types with an equal volume, and grown at an equal density provides an opportunity to compare the fundamentally differing architectural attributes of each while limiting sources of confounding from container volume and density (Pinto et al. 2011a).

This chapter has two experiments. The objective of the first experiment was to examine the effects of container-type and Nitrogen fertilizer application timing post-nursery culture on the post-transplant LWP of koa in a simulated outplanting scenario. The objective of the second experiment of this chapter was to document the effects of container-type and Nitrogen fertilizer application timing on the growth and survival of koa in the field.

Materials and Methods

Experiment 1: Establishment Simulation Study

Koa seeds were collected from a single open-pollinated tree in the Ko‘olau Mountains on O’ahu Island, Hawai’i. Seedlings were grown in an enclosed greenhouse at the Pope Laboratory of the University of Hawai’i at Mānoa in Honolulu, HI (21°18'09.16"N, 157°48'54.42"W) using two container-types. Selected containers were; RootMaker® Express™ 18 containers and Deepot™ D25L containers. Both of these containers have a comparably large volume of 410 cm^3. RootMaker® Express™ 18 containers have a square-shaped top (7.9 × 7.9 cm) and are 10.2 cm deep; they have staggered walls which train roots downward toward holes where they air-prune. Deepot™ D25L containers are tube-like with a 5.1 cm diameter top opening and are 25.4 cm deep; they feature internal ribs that train roots downward and have root pruning holes only at bottom of the container. RootMaker® containers were held in RootMaker® Express™ 18 Injection-molded Trays (RootMaker® Products Company, LLC., Huntsville, AL), and Deepot™ containers were held in Deepot™ D50 support trays (Stuewe and Sons., Inc., Tangent, OR). Containers were uniformly filled with a medium containing a 2:1 (v:v) mixture of sphagnum peat moss and vermiculite.

Both the nursery culture and field simulation components of this experiment followed a completely randomized design (CRD) with a factorial treatment structure (2 fertilizer treatments × 2 container-types × 18 individual seedling replications). Of the 18 replications per treatment, 11 were randomly assigned to be destructively sampled following nursery culture and 7 were randomly assigned to be transplanted for the establishment simulation. Fertilizer treatments consisted of: 1. No reduction in N application after the rapid-growth phase, hereafter referred to as N184 and 2. A reduction in N application following rapid-growth phase, hereafter referred to as H184 (Table 3.1; Figure 3.1). Total applied nitrogen was equal for both fertilizer treatments. Randomization took place at the seedling level. Seedling location was randomized three times per week as to prevent potentially confounding microsite variability.

Nursery Culture

Seed coats were lightly scarified by hand using sand paper. Site of scarification was opposite the side of seed attachment to legume. Great care was given to prevent abrasion to the radical and endosperm. Seeds were allowed to imbibe by submersion in deionized water for 24 hours. After complete imbibition, on 22 August 2015 two seeds were sown per container into RootMaker and Deepot containers at a depth twice the breadth of the seed. During the germination phase, containers were lightly irrigated twice per day using a mist nozzle on a standard garden hose. Germination was considered complete after 15 days, at which time each container was thinned so that only one seedling populated each container. Seedlings were randomly selected within each container-type and randomly assigned a fertilizer treatment using the Microsoft Excel list randomization feature.

Tray irrigation requirement was ascertained by means of the “scientist technique” (Dumroese et al. 2015) of the block-weight method (White and Mastalerz 1966). Irrigation timing was based on gravimetric container weights for each treatment, 65% (±5%) during establishment, rapid-growth, and hardening phases. Nutrients were supplied to individual seedlings as a custom-mixed fertilizer solution. Seedlings were fertigated (fertilizer + irrigation water) three times per week at a rate in accordance to each treatment's respective fertilizer schedule (Table 3.1, 3.2; Figure 3.1). Concentrated stock solutions were used to create custom fertilizer solutions for individual seedlings. Four stock solutions were prepared, and consisted of: 1. Calcium Nitrate, 2. Monopotassium Phosphate/Magnesium Sulfate, 3. Potassium silicate, and 4. General Hydroponics® FloraMicro (General Hydroponics-USA, Santa Rosa, CA). Total nitrogen targets by treatment were: 1. 184 mg N, without reduction in N application after the rapid-growth phase (N184) and 2. 184 mg N, with reduction in N application following rapid-growth phase (H184) (Figure 3.1). Fertilizer solutions were kept at a pH of 6.0 by additions of phosphoric acid or General Hydroponics® pH Up when necessary. To apply nutrients, a 40 mL aliquot of fertilizer solution was precisely and evenly applied by hand, to each seedling. This volume was calculated to bring containers back to field capacity from a 65% dry down. Between fertilization events, seedlings were hand-irrigated with water to field capacity as necessary.

Simulated Field Trials

On 19 November 2015, seedlings representing all treatment combinations were obtained from the first experiment and transplanted into 19 L buckets (21.6 x 38.1 cm) with multiple holes drilled into the bottom for drainage. Buckets were filled with a medium containing a 1:1 (v:v) of coral sand and vermiculite. Buckets were then flushed with water for 1 hour and allowed to drain to 100% field capacity. Seedlings were then transplanted directly into randomly selected buckets. A total of 28 koa seedlings were transplanted into this experiment and grown for 17 days. No additional applications of water were provided. Buckets were housed within the greenhouse and were not affected by precipitation events.

Sampling

On 19 November 2015, at the conclusion of the nursery portion of the experiment, the morphological plant growth characteristics of height (HT) and root-collar diameter (RCD) were measured on all 18 seedlings. Seedling HT was measured from the medium surface to the apical meristem. RCD measurements were taken at the root-collar. A random sample of 11 seedlings were then destructively sampled for root dry mass (RDM), leaf dry mass (LDM), and stem dry mass (SDM) per treatment. First, root systems were carefully washed free of all media. Second, seedlings were severed at the root-collar and roots, leaves, and stems were bagged separately and dried at 60°C for 72 hours. Shoot dry mass (SHDM) was calculated by summing LDM and SDM. Following drying, RDM, LDM, and SDM were measured and subsequently used to assess seedling root-to-shoot ratio (R:S). The remaining 7 seedlings were randomly assigned to be transplanted into the field simulation experiment.

To determine plant water status of seedlings in the simulated field trial, leaf water potential (Ψ_l) was measured before sunrise (0400; Ψ_{pd}) and at mid-day (1200; Ψ_{md}) 5, 12, and 17 days after transplant. Ψ_l was measured with a Model 1000 Pressure Chamber Instrument (PMS Instrument Company, Albany, OR). Only sunny days were chosen for measurement to avoid confounding light levels. To measure Ψ_l, a healthy fully-expanded leaf near the top of each tree was selected for sampling. To minimize damage to trees, leaflets of true leaves were used to determine Ψ_l instead of entire leaves. A test was performed on leaflets to determine their suitability for measuring koa Ψ_l compared to full leaves; Ψ_l readings of leaves and leaflets were performed on the same individual and replicated across 5 test seedlings (same seed source but not associated with this study). Readings were indistinguishable between leaves and leaflets. For the experiment, leaflets were carefully severed at the junction with the leaf petiole and immediately taken to the pressure chamber. Leaflets were then gently secured into the chamber lid, and the Ψ_l reading was taken. This process was repeated for every tree.

Statistical Analysis

For each response variable, normal quantile plots were constructed for each treatment and the pooled residuals from all treatments to assess normality. A Brown-Forsythe test was ran for each response variable to determine the equal variance assumption for an analysis of variance. Data met all assumptions for normality and equal variance, and thus no transformations were necessary. An analysis of variance was used to analyze fertilizer and container main effects of plant growth characteristics and Ψ_l after nursery culture, destructive harvest, and simulated outplanting trial. Subsequent interactions, if any, were analyzed using a standard least-squares fit model with a level of significance at α=0.05 for each response variable. The model included the main effects of container-type and fertilizer treatment as well as their interaction. Analyses were performed using SAS JMP Pro Statistical Software (Version 12.0).

Experiment 2: Field Trials

Nursery Culture

Seedlings for the outplanting experiment were grown using the same methods as in Experiment 1. The same CRD structure was used with 6 replications. Seeds were sown on October 22, 2015 two seeds were sown per container into 150 RootMaker and 150 Deepot containers. Germination was considered complete after 14 days, at which time each container was thinned so that only one seedling populated each container. Containers in which no seeds germinated were removed. Of the remaining seedlings, 108 were randomly selected per container-type and randomly assigned a fertilizer treatment, tray, and location in tray.

Fertilizer treatments were carried out as in Experiment 1, but the rates were changed, and potassium silicate was not utilized. Total nitrogen targets by treatment were: 1. 172 mg N, without reduction in N application after the rapid-growth phase

(N172) and 2. 172 mg N, with reduction in N application following rapid-growth phase (H172) (Table 3.3, 3.4; Figure 3.2).

Field Site and Study Layout

The selected field site is located above the Waimea Valley on the North Shore of O'ahu (21°37'52.36"N, 158°02'08.96"W), is owned by the Office of Hawaiian Affairs, and is managed by Hiipaka LLC. The field slope is relatively flat, has a south aspect, and is 203 m in elevation. Soils formed in alluvium and colluvium from basic igneous rock of the Helemano series (classified as very-fine, kaolinitic, isohyperthermic Rhodic Eutrustox) (NRCS 2016). Helemano soils are characterized as very deep, well-drained soils with a silty clay texture and a neutral pH of 6.5 (NRCS 2016). Precipitation records from a 33 year period in the vicinity of the field site in Pupukea average 1585 mm annually (Giambelluca et al. 2013). The average annual temperature is 22.6 °C; the coldest month is Feb with an average of 20.7 °C; the hottest month is Aug with an average of 24.4 °C (Giambelluca et al. 2014). The land cover is classified as a Hawaiian lowland mesic forest and Hawaiian introduced perennial grassland. Site is predominantly covered by alien species such as; molasses grass (*Melinis minutiflora*), *Clidemia hirta*, java plum (*Syzygium cumini*), formosa koa (*Acacia confusa*), and strawberry guava (*Psidium cattleianum*). Mature and declining populations of *Acacia koa* and Lama (*Diospyros sandwicensis*) can be found sparsely spread in the vicinity of the site.

Site Preparation and Planting

The experimental site was fenced with a Patriot Solarguard 50 0.05 J electric fence energizer at a height of 10, 20, 45, and 75 cm to exclude feral hogs, piglets, goats, and kids. On 28 December 2015, 0.66 L of Ranger® Pro (EPA reg. no. 524-517, Monsanto Company, St. Louis, MO) was added to 30 L of water with an organosilicone surfactant adjuvant. Herbicide mixture was evenly applied to 325 m^2 at the experimental site using a backpack sprayer with a brass cone adjustable nozzle. The heavy herbicide application (7.23 kg glyphosate a.e. ha^{-1}) suppressed all grass competition on-site by the time of planting. The outplanting operation was conducted over a 10 hour time period on 28 January 2016. To avoid planter technique confounding, all seedlings were hand-planted by one individual using a gas-powered 100 mm diameter auger. At the completion of nursery culture, seedlings from each treatment were randomly selected, randomly assigned a block, and randomly assigned a planting location in the block. Experimental site held 4 blocks with each block having 36 experimental seedlings representing 4 nursery culture treatments with 9 individuals from each treatment present in every block. Plant spacing was 1 m, with a minimum of 2 m between blocks.

Sampling

At the conclusion of nursery culture when seedlings were 13 weeks old, on 26 January 2016, seedling morphological growth characteristics were measured using the same methodology as Experiment 1. Measurements of height HT and RCD were measured on all seedlings at lifting. RDM, LDM, and SDM were measured on a randomly selected subset of 2 seedlings per tray replication. SHDM and R:S were calculated as in Experiment 1.

On the day of planting, seedling HT and RCD were measured. HT and RCD along with survivorship was measured at 2, 4, and 8 months after planting. HT was measured from the ground-line to the apical meristem; RCD was measured approximately 1 cm above ground-line. Soil edaphic measurements were collected in each block. Soil volumetric water content (θ) and was measured at depths of 10, 18, and 26 cm. Soil temperature was measured at a depth of 18 cm. Measurements were collected hourly using EC-5 (10 & 26 cm deep) and ECH_2O-TE soil moisture sensors connected to EM50 data loggers (Decagon Devices, Inc., Pullman, WA).

Statistical Analysis

For each response variable, normal quantile plots were constructed for each treatment and the pooled residuals from all treatments to assess normality. A Brown-Forsythe test was ran for each response variable to determine the equal variance assumption for an analysis of variance. All data met all assumptions for normality and equal variance, and thus no transformations were necessary. An analysis of variance was used to analyze fertilizer and container main effects of plant growth characteristics after nursery culture. Subsequent interactions, if any, were evaluated using the Tukey HSD test; differences were deemed significant at (α=0.05) for each response variable. The model included the main effects of container-type and fertilizer treatment as well as their interaction. A mixed model ANOVA was used to analyze outplanting data 8 months after planting; block was classified as the random variable. Subsequent interactions, if any, were evaluated using the Tukey HSD test; differences were deemed significant at (α=0.05) for each response variable. Analyses were performed using SAS JMP Pro Statistical Software (Version 12.0).

Results

Experiment 1: Establishment Simulation Study

Nursery Culture

For the morphological response variables measured, interactions between container-type × fertilizer treatments were not significant ($p > 0.1077$) (Table 3.5). Thus, each treatment main effect was considered independently when evaluating response variables. Morphological characteristics differed between treatments in response to container-type and fertilizer treatment (Table 3.5). The main effect of container-type significantly influenced HT, RCD, RDM, and R:S ($p < 0.0001$) while fertilizer treatments significantly influenced HT, SDM, LDM, and SHDM ($p \leq 0.0367$).

Deepot containers produced a significantly greater HT than RootMaker containers (+7%; p = 0.0003). H184 seedlings had a significantly decreased HT compared to N184 seedlings (-6%; p = 0.0018). RootMaker containers produced a significantly greater RCD than Deepot containers (+10%; $p < 0.0001$). A low-N hardening phase had no effect on RCD (p = 0.3817).

Deepot containers produced a significantly greater R:S than RootMaker containers (+50%; $p < 0.0001$). A low-N hardening phase did not significantly affect the R:S of seedlings (p = 0.3214). Deepot containers also produced a significantly greater RDM compared to RootMaker containers ($p < 0.0001$). A low-N hardening phase had no effect on RDM (p = 0.9481).

H184 seedlings had a significantly decreased SDM (-8%), LDM (-10%), and SHDM (-10%) compared to N184 seedlings ($p < 0.0330$). Container-type had no significant effect on SDM, LDM, and SHDM ($p > 0.5368$).

Simulated Field Trials

For the measures of Ψ_{pd} and Ψ_{md} measured 5, 12, and 17 days after transplant, interactions between container-type × fertilizer treatments were not significant ($p >$ 0.1047) (Table 3.6). Thus, each treatment main effect was considered independently when evaluating response variables. Ψ_l differed between treatments in response to container-type and fertilizer treatment (Table 3.6).

Five days after transplant, container-type and fertilizer treatment significantly influenced Ψ_{md}. RootMaker seedlings were under decreased water stress compared to Deepot seedlings (-23%; $p < 0.0001$) and H184 seedlings were under decreased water stress compared to N184 seedlings (-20%; $p < 0.0027$). Container-type significantly affected Ψ_{pd} with RootMaker seedlings experiencing 18% less water stress than Deepot seedlings (p = 0.0074). Fertilization had no effect on Ψ_{pd} (p = 0.2716).

Twelve days after transplant, container-type and fertilizer treatment had no significant effect on Ψ_{md} ($p > 0.1703$). H184 seedlings had a 13% increased Ψ_{pd} compared to N184 seedlings ($p = 0.0360$). Container-type had no significant effect on Ψ_{pd} ($p = 0.5381$).

Seventeen days after transplant, container-type significantly influenced Ψ_{md} ($p = 0.0025$), while the fertilizer treatment did not ($p = 0.9321$). RootMaker containers were under 35% increased water stress compared to Deepot containers ($p = 0.0025$). No significant difference was detected between fertilizer treatments ($p = 0.9146$). H184 seedlings had a 23% increased Ψ_{pd} compared to N184 seedlings ($p = 0.0151$). Container-type had no significant effect on Ψ_{pd} ($p = 0.1053$).

Experiment 2: Field Trials

Nursery Culture

For the morphological response variables measured, interactions between container-type and fertigation treatment were significant for LDM, SHDM, and RDM ($p < 0.0082$) (Table 3.7). The main effect of container-type significantly influenced HT, RCD, SDM, and R:S ($p < 0.0425$) while fertilizer treatment significantly influenced RCD and R:S ($p < 0.0294$).

In contrast to previous experiments, a low-N hardening phase did not result in a significant decrease in seedling HT compared to unhardened seedlings ($p = 0.9729$). This anomalous lack of a decrease in N hardened seedling HT implicated subsequent increases of SDM and LDM and could explain the source of interaction significance within LDM and SHDM. Deepot H172 had a decreased LDM compared to Deepot N172 (2.90±0.15 vs. 3.59±0.17 g; $p = 0.0154$). While there was no significance difference in LDM between RootMaker H172 and N172 ($p = 0.7223$). Deepot H172 had a decreased SHDM compared to Deepot N172 (4.26±0.21 vs. 5.19±0.20 g; $p = 0.0109$). While there was no statistical significance in SHDM between RootMaker H172 and N172 (p = 0.7224). RootMaker H172 had an expected result of a significantly increased RDM compared to RootMaker N172 (1.32±0.04 vs. 1.07±0.03 g) (p =0.0010). The significant interaction in RDM can be attributed to the RDM of Deepot seedlings differing from expectations and previous experiments by having a non-significant difference in RDM between H172 and N172 treatments (1.32±0.03 vs. 1.37±0.05 g; p =0.8245). Interestingly, these interactions did not implicate an interaction in the R:S response (p = 0.4521).

RootMaker containers produced a significantly greater HT than Deepot containers (12%; $p < 0.0001$). RootMaker seedlings had a significantly greater RCD than Deepot seedlings (12%; $p < 0.0001$). A low-N hardening phase significantly increased the R:S of seedlings (22%; $p = 0.0183$). Deepot containers also produced a significantly greater R:S than RootMaker containers (32%; $p < 0.0001$). RootMaker containers produced a significantly greater SDM than Deepot containers (19%; $p < 0.0079$).

For the measured response variable of final 2 week water use, interactions between container-type × fertilizer treatments were not significant ($p < 0.2960$) (Table 3.8). Thus, each treatment main effect was considered independently. The main effects of container-type and fertilizer treatment significantly influenced water use ($p < 0.0214$) (Table 3.8). RootMaker seedlings required a significantly increased quantity of water (15%; $p < 0.0001$). H172 seedlings required a significantly decreased quantity of water (-4%; $p < 0.0214$).

Field Trials

For the morphological response variables measured, interactions between container-type and fertilizer treatments were not significant (Table 3.9). Thus, each treatment main effect was considered independently when evaluating response variables. Morphological characteristics differed between treatments in response to container-type and fertilizer treatment (Table 3.9). The main effect of container-type significantly influenced HT and RCD ($p < 0.0033$) while fertilizer treatment did not significantly influence 8 month morphology ($p > 0.2327$). Neither container-type nor fertilizer treatment significantly influenced 8 month field survival ($p = 0.2932$). Total seedling survival after 8 months of field growth was high (>95%).

Deepot containers produced a significantly greater HT than RootMaker containers after 8 months of field growth (9%; $p = 0.0033$) (Figure 3.3). No significant differences were detected with regard to fertilizer treatment and seedling HT ($p = 0.3579$). Deepot containers produced a significantly greater RCD than RootMaker containers after 8 months of field growth (12%; $p = 0.0002$) (Figure 3.4). No significance was detected in regard to fertilizer treatment and seedling RCD ($p = 0.2643$).

Discussion

Experiment 1: Establishment Simulation Study

These studies tested the hypotheses that reducing the amount of applied nitrogen in the final four weeks of nursery culture (N hardening) would decrease height growth and increase the R:S of koa, and that the subsequent morphology of seedlings hardened in this manner would have an increased proclivity to survive when outplanted into moisture-limited soils. These studies also compared seedlings grown in two container-types with an equal volume and grown at an equal density, but with fundamentally differing architectural attributes. Studies have shown growth and survival benefits of stock grown in deeper containers (Amidon et al. 1982; Rose et al. 1997; Chirino et al. 2008), and air-pruned containers have been shown to create a fibrous root system (Larson 1975). Avoiding the confounding related issues by having an equal planting density and volume allowed for a useful comparison of stocktype produced from these containers.

In the establishment simulation study, H184 seedlings initially had a decreased Ψ_l compared to N184 (Table 3.6). This is could be due to H184 seedlings requiring less water at the time of planting compared to N184 seedlings (Table 3.8). The experiment in chapter two and (Trubat et al. 2008) found that N hardened seedlings had a diminished foliar N concentration. While foliar N data was not collected, it stands to reason that N hardened seedlings in this experiment could also have diminished foliar N reserves. There is a strong positive correlation between leaf N concentration and photosynthetic capacity (Field and Mooney 1986; Reich et al. 1997; Reich et al. 1999). A reduced photosynthetic capacity could have influenced the significantly lower 5 day Ψ_l measures of H184 seedlings as photosynthetic capacity is correlated to stomatal conductance and water demand (Chapin et al. 2002). However, this possible pattern of decreased water use at the end of nursery culture translating into a reduced soil water requirement post-transplant was not found between container-types. RootMaker seedlings initially had a decreased Ψ_l compared to Deepot seedlings (Table 3.6) while requiring more water in nursery culture (Table 3.8).

The differential structural nature of stocktype produced by these containers could explain the Ψ_l differences of these seedlings through time. Consider the Ψ_l of treatments as time progresses after transplant, Ψ_l decreases in Deepot seedlings, and increases in RootMaker seedlings (Table 3.6). It is possible that the inherently deeper taproot and root tips in the Deepot stocktype allowed these seedlings to acquire an increased amount of water relative to RootMakers, and recover rapidly. Studies have shown that facilitating taproot growth with deep containers gives outplanted seedlings an advantage in accessing deep soil moisture (Canadell et al. 1996; Chirino et al. 2008). Deepot seedlings had an architecturally advantageous root structure by having root tips at considerably more soil depth levels than RootMaker seedlings, and could have subsequently explored the bucket containers more conveniently.

In addition, RootMaker seedlings, with their initial root structure being exclusively near the soil surface, were positioned in the bucket where soil water losses from evaporation would originate, and from their own exploitation of near-surface water reserves. The nature of the fibrous root system produced by RootMaker containers could have also been antithetical to their ability to explore deeper soil water reserves. Fibrous root systems are typically short-lived, and are primarily constructed for the purpose of water and nutrient absorption, and do not typically undergo secondary growth (Polverigiani et al. 2011). This primary purpose of water absorption could also be the cause of their reduced Ψ_l compared to Deepot seedlings initially after transplanting. Construction of pioneer roots to explore deeper soil stratums comes at a higher construction cost per unit length (Guo et al. 2004; Huang et al. 2010) that N-deprived seedlings would be burdened to afford. Initial benefits in soil water absorption from fibrous RootMaker seedling roots could deteriorate over time when those adjacent water reserves diminish, and the plant needs to produce pioneer roots to allocate further water resources. A low-N hardening phase could have exhausted translocatable nutrients in seedlings and led to root systems which could not egress sufficiently in order to more effectively combat water stress.

Experiment 2: Field Trials

In contrast to Trubat and others (2008) which found increased survival of Mediterranean species planted in an arid environment, a low-N hardening phase did not confer survival benefits to outplanted koa seedlings. Seedling survival in the field was high in all treatments (>95%). This is an interesting and promising result considering that these seedlings were planted during the droughty 2016 El Niño season, and established without any additions of water for 18 days following planting (Figure 3.5). Soil moisture data shows that site volumetric soil moisture measures were relatively high at all measured depths (Figure 3.5). Site Helemano series soils (NRCS 2016), with a silty-clay texture have the capacity to hold a large quantity of plant available soil water (Weil and Brady 2016). High volumetric water levels likely diminished the effect of soil-moisture as a limiting factor at this site. Soil temperate at 18 cm was also within a favorable range during the 8 months of field growth (Figure 3.6). High survival may also be attributed to the decision to utilize relatively large containers (410 cm^3) and follows what has been documented of koa grown in larger containers in Hawai'i (Dumroese et al. 2011; Pinto et al. 2015).

Increased field growth accrued by Deepot containers may be due to a diminished relative water stress as documented in the establishment simulation study. At the completion of that study, Deepot seedlings had a 35% lower Ψ_l reading than RootMaker seedlings (Table 3.6). With the evidence that Deepot stocktype experience reduced water stress compared to RootMaker stocktype, it stands to reason that Deepot seedlings would have a proclivity to initiate the positive feedback loop of seedling establishment more rapidly than RootMaker seedlings. The fibrous nature of RootMaker seedling roots could have put them at a structural disadvantage in having predominantly fibrous roots which do not have the capacity to explore soil horizons as effectively as pioneer roots (Polverigiani et al. 2011). Deepot seedlings had an architecturally advantageous root structure by having root tips at considerably more soil depth levels than RootMaker seedlings, and could have subsequently explored more soil horizons. Many studies have found seedlings grown in larger and deeper containers outperform more shallow containers in the field (Amidon et al. 1982; Rose et al. 1997; Chirino et al. 2008). Deepot root systems are 150% deeper than RootMaker root systems. The increased root system depth coupled with not having a potentially disadvantageous amount of near-surface fibrous roots (Polverigiani et al. 2011), could have influenced their ability to access increased soil water and nutrient reserves and grow at relatively increased rate.

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Tables

Table 3.1. Experiment 1 nutrient application quantities (mg) per seedling per application event by treatment and growth-phase.

	H184			N184	
Nutrient	**Establishment**	**Rapid-Growth**	**Hardening**	**Establishment**	**Rapid-Growth**
N	2.0	9.69	3.0	2.0	7.18
P	0.64	4.0	4.0	0.64	4.0
K	1.2	6.74	6.74	1.2	6.74
Ca	0.4	8.80	0.6	0.4	5.12
Mg	0.64	2.54	2.54	0.64	2.54
S	0.83	3.30	3.30	0.83	3.30
Fe	0.06	0.06	0.06	0.06	0.06
Mn	0.03	0.03	0.03	0.03	0.03
Zn	0.009	0.009	0.009	0.009	0.009
Cu	0.004	0.004	0.004	0.004	0.004
Mo	0.00048	0.00048	0.00048	0.00048	0.00048
Co	0.0003	0.0003	0.0003	0.0003	0.0003
Si	0.0	1.0	1.0	0.0	1.0

Table 3.2. Experiment 1 fertilization event dates by treatment and growth-phase September through November, 2015.

Establishment Phase: H184, N184

9/6	9/8	9/10	9/13	9/15	9/17

Rapid-Growth Phase: H184, N184

9/20	9/22	9/24	9/27	9/29	10/1	10/4	10/6	10/8	10/11	10/13	10/15	10/18	10/20	10/22

Hardening Phase: H184 / Rapid-Growth Phase: N184

10/25	10/27	10/29	11/1	11/3	11/5	11/8	11/10	11/12

Table 3.3. Experiment 2 nutrient application quantities (mg) per seedling per application event by treatment and growth-phase.

	H172			N172	
Nutrient	**Establishment**	**Rapid-Growth**	**Hardening**	**Establishment**	**Rapid-Growth**
N	2.0	8.0	2.0	2.0	5.5
P	0.64	3.32	3.32	3.32	3.32
K	1.2	4.8	4.8	1.2	4.8
Ca	0.4	7.75	0.4	0.4	4.69
Mg	0.64	1.8	1.8	0.64	1.8
S	0.83	2.34	2.34	0.83	2.34
Fe	0.04	0.04	0.04	0.04	0.04
Mn	0.02	0.02	0.02	0.02	0.02
Zn	0.006	0.006	0.006	0.006	0.006
Cu	0.004	0.004	0.004	0.004	0.004
Mo	0.00032	0.00032	0.00032	0.00032	0.00032
Co	0.0002	0.0002	0.0002	0.0002	0.0002

Table 3.4. Experiment 2 fertilization event dates by treatment and growth-phase November 2015 through January 2016.

Establishment Phase: H172, N172

11/5	11/8	11/10	11/12	11/15	11/17

Rapid-Growth Phase: H172, N172

11/19	11/22	11/24	11/26	11/29	12/1	12/3	12/6	12/8	12/10	12/13	12/15	12/17	12/20	12/22	12/24	12/27

Hardening Phase: H172 / Rapid-Growth Phase: N172

12/29	12/31	1/3	1/5	1/7	1/10	1/12	1/14	1/17	1/19	1/21	1/24

Table 3.5. Experiment 1 morphological characteristics and sources of variation of *Acacia koa* seedlings following 12 weeks of nursery culture. Statistical means for measured values and the associated standard error (height and root-collar diameter n=68; all other measures n=44). Main effects treatments are: 1) Containers: RootMaker or Deepot, and 2) Fertilizer: H184-reduction in N application following rapid-growth phase (184 mg N seedling^{-1}); N184-no reduction in N application following rapid-growth phase (184 mg N seedling^{-1}).

Treatment	Height (cm)		Root-Collar Diameter (mm)		Root-to-Shoot Ratio		Root Dry Mass (g)		Stem Dry Mass (mm)		Leaf Dry Mass (g)		Shoot Dry Mass (g)	
Container (C)														
RootMaker	**47.80(0.55)**	**a**†	**5.42(0.04)**	**a**	**0.14(0.006)**	**a**	**0.74(0.04)**	**a**	1.47(0.05)	a	3.67(0.10)	a	5.18(0.13)	a
Deepot	**51.23(0.70)**	**b**	**4.94(0.06)**	**b**	**0.21(0.008)**	**b**	**1.10(0.05)**	**b**	1.50(0.04)	a	3.76(0.10)	a	5.23(0.14)	a
Fertilizer (F)														
H184	**48.03(0.63)**	**a**	5.23(0.06)	a	0.18(0.01)	a	0.91(0.06)	a	**1.42(0.04)**	**a**	**3.52(0.07)**	**a**	**4.94(0.10)**	**a**
N184	**50.99(0.66)**	**b**	5.15(0.06)	a	0.17(0.01)	a	0.92(0.06)	a	**1.55(0.04)**	**b**	**3.91(0.11)**	**b**	**5.46(0.14)**	**b**
Source of Variation														
C	**<0.0001***		**<0.0001***		**<0.0001***		**<0.0001***		0.5695		0.5036		0.7634	
F	**0.0004***		0.2134		0.1645		0.9321		**0.0367***		**0.0052***		**0.0049***	
C×F	0.1077		0.2769		0.5968		0.6913		0.8039		0.4570		0.5193	

*Indicates significance (α=0.05).

†Different letters indicate significant differences within column for each response variable (α=0.05).

Table 3.6. Experiment 1 pre-dawn (PD) (0400) and mid-day (MD) (1200) *Acacia koa* seedling leaf water potential (MPa) and sources of variation 5,12, and 17 days after transplant. Statistical means for measured values and the associated standard error (n=28).

Treatment	5 Days MD		12 Days MD		17 Days MD		5 Days PD		12 Days PD		17 Days PD	
Container (C)												
RootMaker	**-1.18(0.06)**	**a**[†]	-1.16(0.04)	a	**-1.44(0.09)**	**a**	**-0.32(0.02)**	**a**	-0.40(0.02)	a	-0.47(0.03)	a
Deepot	**-1.54(0.05)**	**b**	-1.14(0.04)	a	**-1.07(0.06)**	**b**	**-0.39(0.02)**	**b**	-0.42(0.01)	a	-0.40(0.02)	a
Fertilizer (F)												
H184	**-1.21(0.07)**	**a**	-1.09(0.06)	a	-1.26(0.10)	a	-0.34(0.02)	a	**-0.43(0.02)**	**a**	**-0.48(0.03)**	**a**
N184	**-1.51(0.05)**	**b**	-1.20(0.05)	a	-1.25(0.08)	a	-0.37(0.02)	a	**-0.38(0.01)**	**b**	**-0.39(0.02)**	**b**
Source of Variation												
C	**<0.0001***		0.7865		**0.0025***		**0.0069***		0.4941		0.0706	
F	**<0.0001***		0.1836		0.8976		0.2116		**0.0317***		**0.0106***	
C×F	0.5053		0.5289		0.1656		0.2804		0.1047		0.3647	

*Indicates significance (α=0.05).

[†]Different letters indicate significant differences within column for each response variable (α=0.05).

Table 3.7. Experiment 2 morphological characteristics and sources of variation of *Acacia koa* seedlings following 13 weeks of nursery culture. Statistical means for measured values and the associated standard error (n=24). Main effects treatments are: 1) Containers: RootMaker or Deepot, and 2) Fertilizer: H172-reduction in N application following rapid-growth phase (172 mg N $seedling^{-1}$); N172-no reduction in N application following rapid-growth phase (172 mg N $seedling^{-1}$).

Treatment	Height (cm)		Root-Collar Diameter (mm)		Root-to-Shoot Ratio		Root Dry Mass (g)		Stem Dry Mass (mm)		Leaf Dry Mass (g)		Shoot Dry Mass (g)	
Container (C)														
RootMaker	**49.78(0.62)**	**a**†	**5.84(0.05)**	**a**	**0.22(0.008)**	**a**	1.19(0.05)	a	**1.75(0.06)**	**a**	3.58(0.10)	a	5.33(0.12)	a
Deepot	**44.53(0.68)**	**b**	**5.22(0.03)**	**b**	**0.29(0.01)**	**b**	1.35(0.03)	a	**1.47(0.06)**	**b**	3.25(0.15)	a	4.72 (0.20)	a
Fertilizer (F)														
H172	47.18(1.24)	a	5.61(0.11)	a	**0.28(0.01)**	**a**	1.32(0.02)	a	1.57(0.08)	a	3.29(0.16)	a	4.86(0.23)	a
N172	47.13(0.75)	a	5.45(0.09)	a	**0.23(0.01)**	**b**	1.22(0.05)	a	1.66(0.07)	a	3.53(0.10)	a	5.19(0.12)	a
Source of Variation														
C	**<0.0001***		**<0.0001***		**<0.0001***		**0.0007***		**0.0066***		**0.0341***		**0.0042***	
F	0.9559		**0.0024***		**0.0002***		**0.0160***		0.3418		0.1192		0.0974	
C×F	0.0826		0.2324		0.4521		**0.0010***		0.1150		**0.0055***		**0.0042***	

*Indicates significance (α=0.05).

†Different letters indicate significant differences within column for each response variable (α=0.05).

Table 3.8. Experiment 2 sources of variation and *P* values observed for *Acacia koa* seedling total water use during the final 2 weeks of nursery culture (12 January to 26 January 2016). Response to container-type, fertilizer treatment, and their interaction. Statistical means for measured values and the associated standard error (n=24).

	Total Water Use per Seedling (mL)	
Container (C)		
RootMaker	**1176(11)**	**a**
Deepot	**1024(16)**	**b**
Fertilizer (F)		
H172	**1077(28)**	**a**
N172	**1122(24)**	**b**
Source of Variation		
C	**<0.0001***	
F	**0.0214***	
C×F	0.2960	

*Indicates significance (α=0.05).
†Different letters indicate significant differences within column for each response variable (α=0.05).

Table 3.9. Experiment 2 sources of variation and *P* values observed for *Acacia koa* seedling survival and morphological growth response to container-type, fertilizer treatment, and their interaction after 8 months of growth in the field (survival n=144; height and root-collar diameter n=119).

	Container	Fertilization	Container × Fertilization
Survival	0.2932	0.2932	0.7184
Height	**0.0033***	0.3316	0.2863
Root-Collar Diameter	**0.0002***	0.2327	0.2791

*Indicates significance (α=0.05).

Figures

Figure 3.1. Schematic of experiment 1 fertilizer treatment schedules by macronutrient (N,P,K).

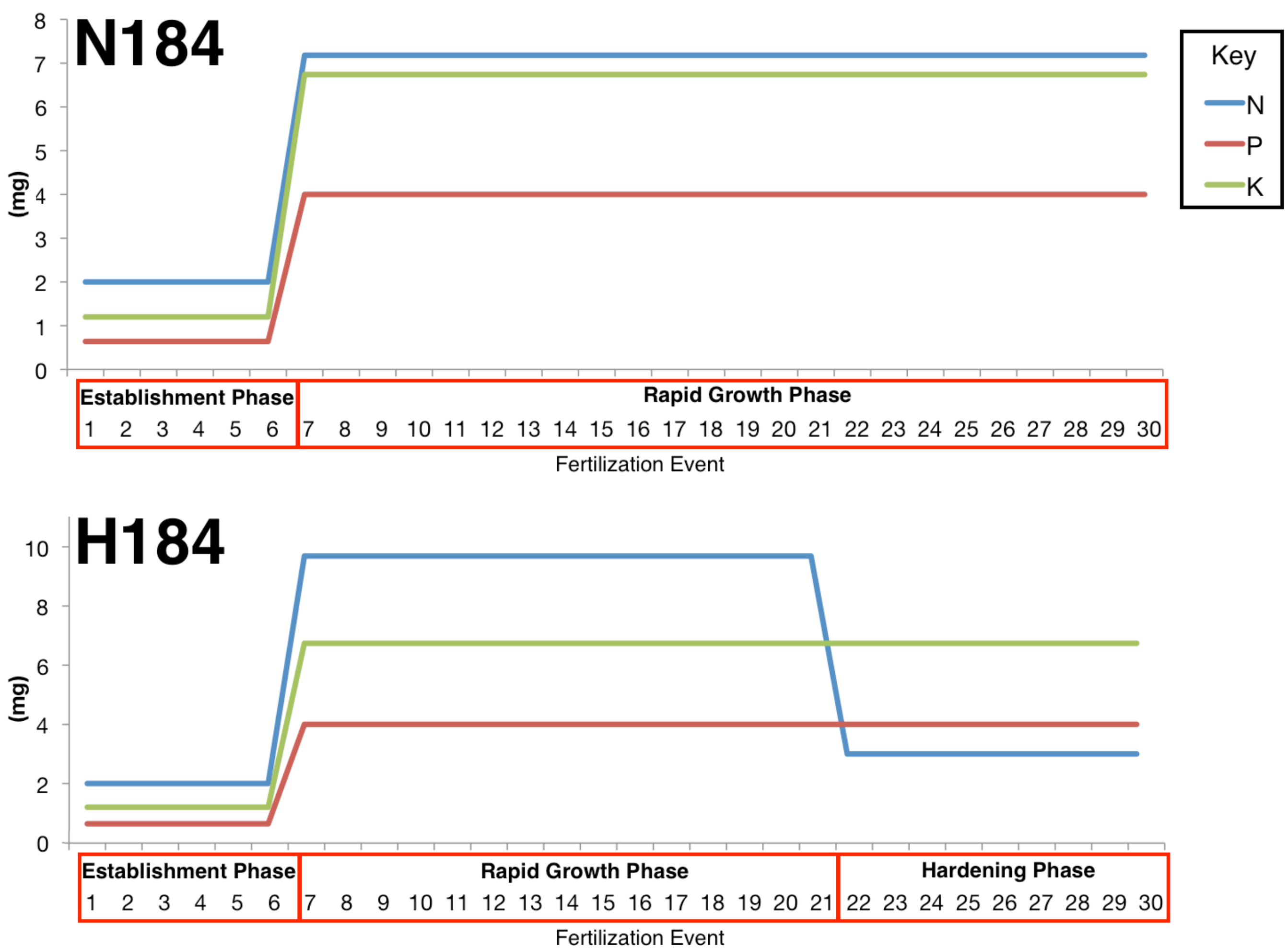


Figure 3.2. Schematic of experiment 2 fertilizer treatment schedules by macronutrient (N,P,K).

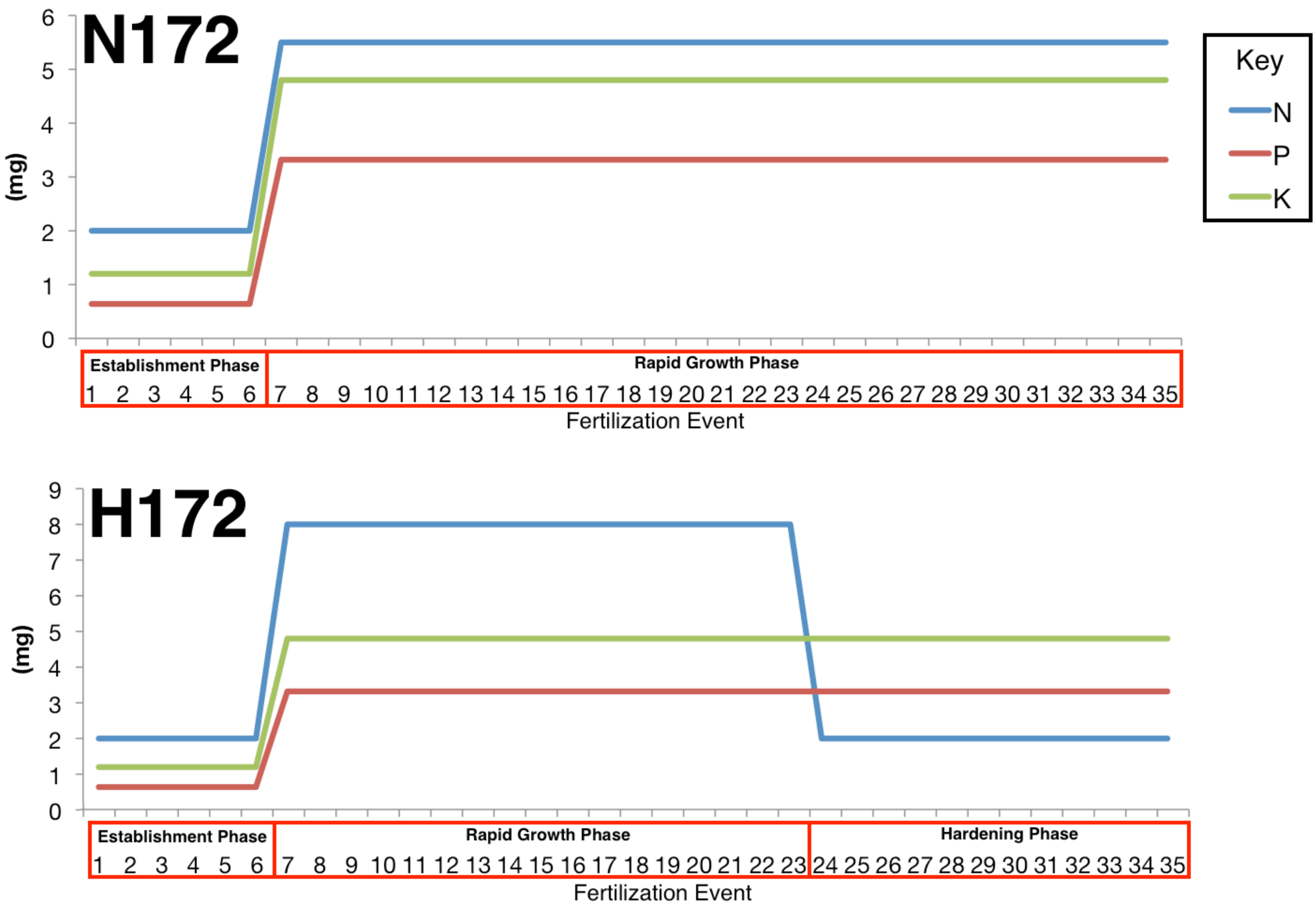


Figure 3.3. Mean (±SE) height following 8 months of growth in the field (n=119). Different letters indicate significant differences (α=0.05).

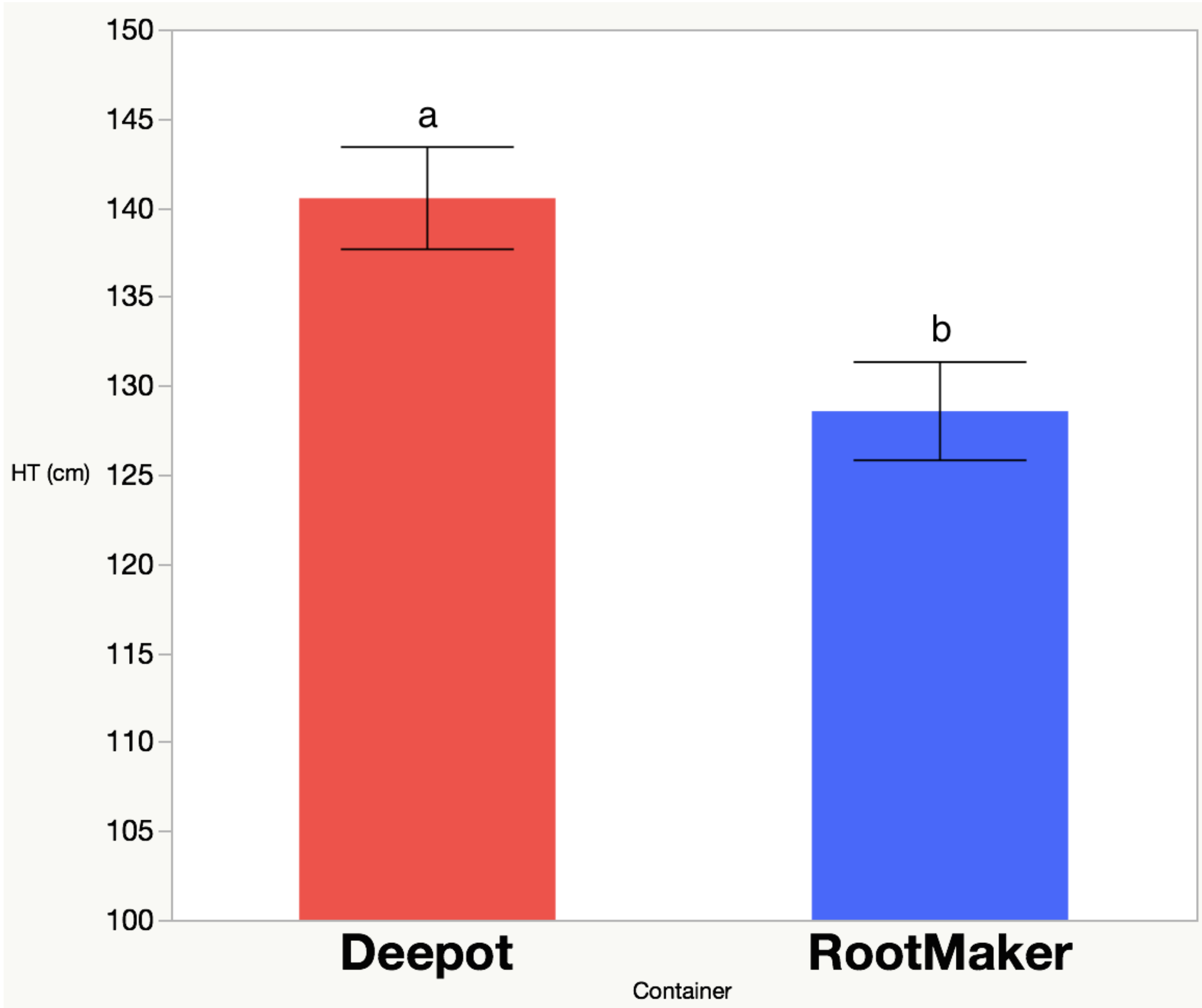


Figure 3.4. Mean (±SE) root-collar diameter following 8 months of growth in the field (n=119). Different letters indicate significant differences (α=0.05).

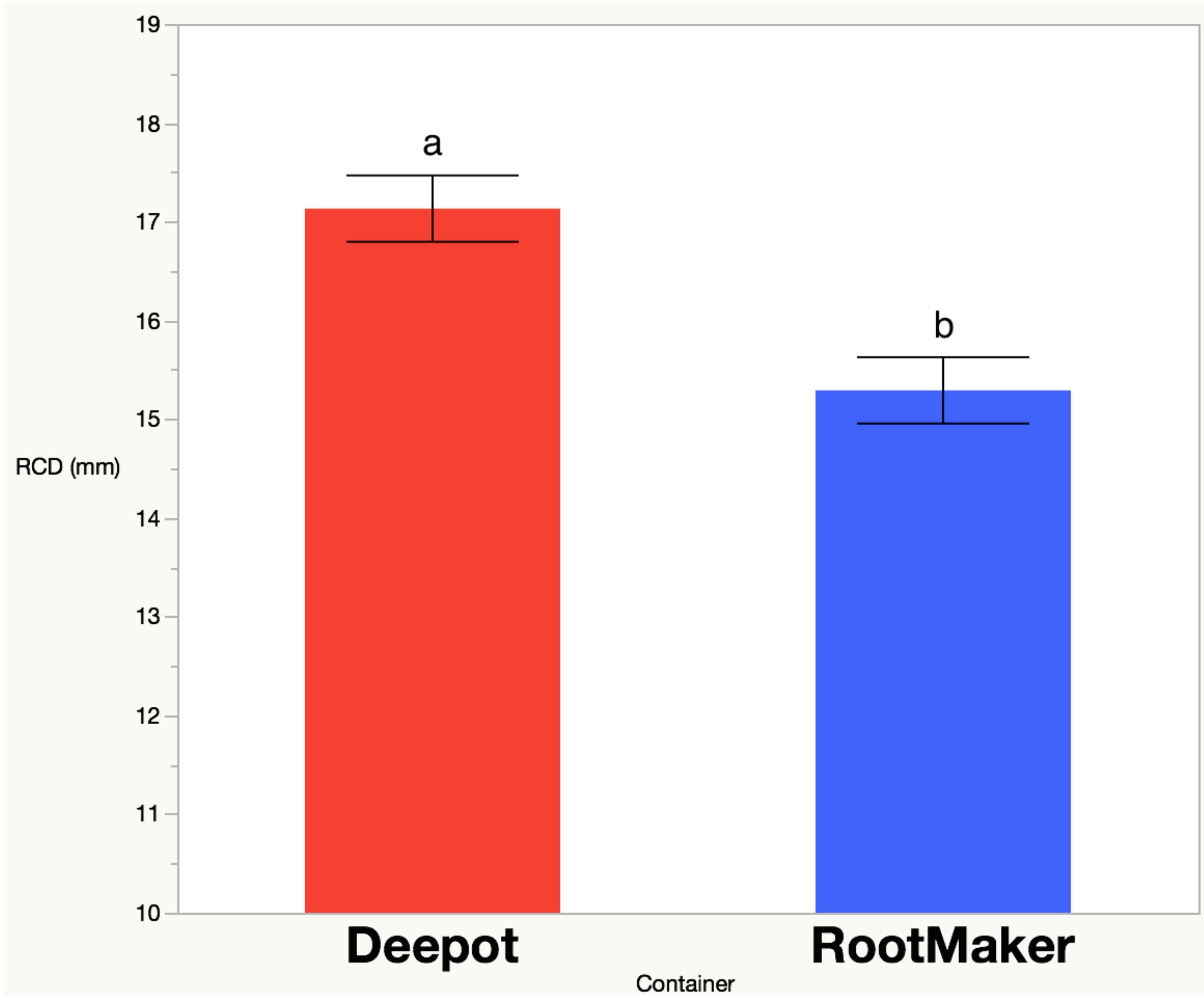


Figure 3.5. Mean field site soil volumetric water content m³ m⁻³ at 10, 18, and 26 cm depth through time (n=4).

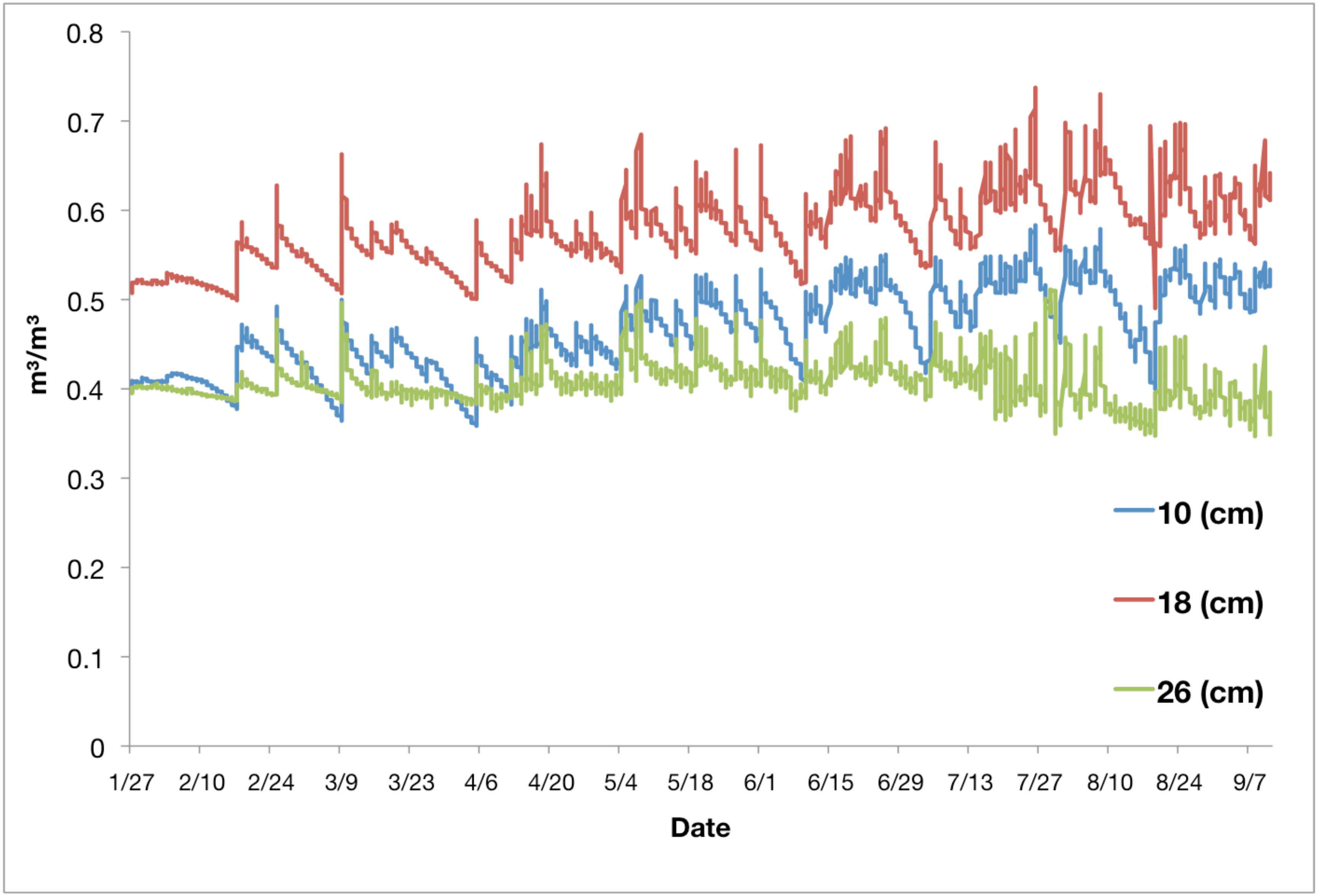
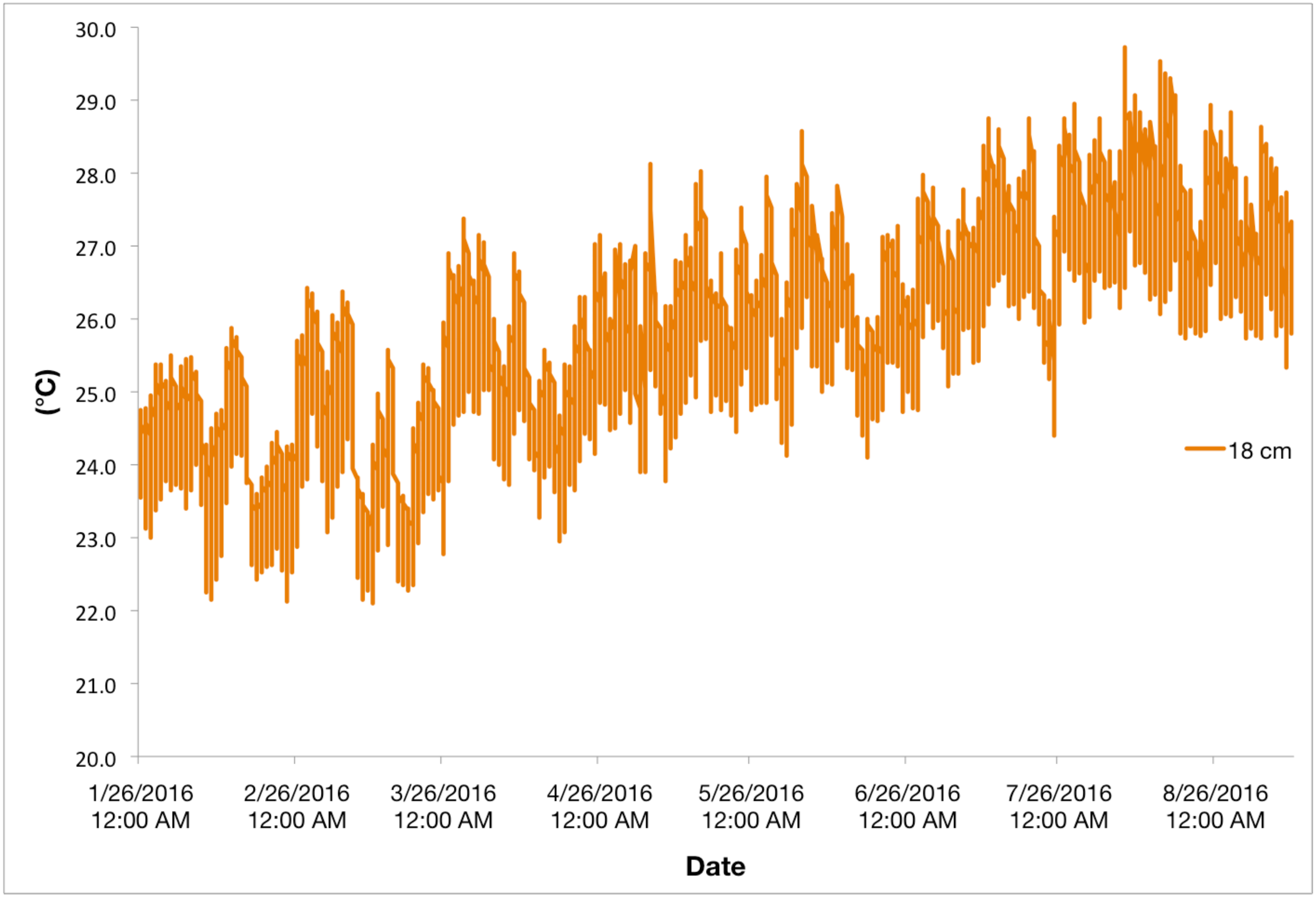


Figure 3.6. Mean field site soil temperature (°C) at 18 cm depth through time (n=4).



Images

Image 3.1. Work station to measure gravimetric water content and fertilize *Acacia koa* seedlings.

Image 3.2. *Acacia koa* seedlings at the initiation of the establishment simulation in experiment 1 (20 November 2015).

Image 3.3. Work station to measure leaf water potential in the establishment simulation in experiment 1.

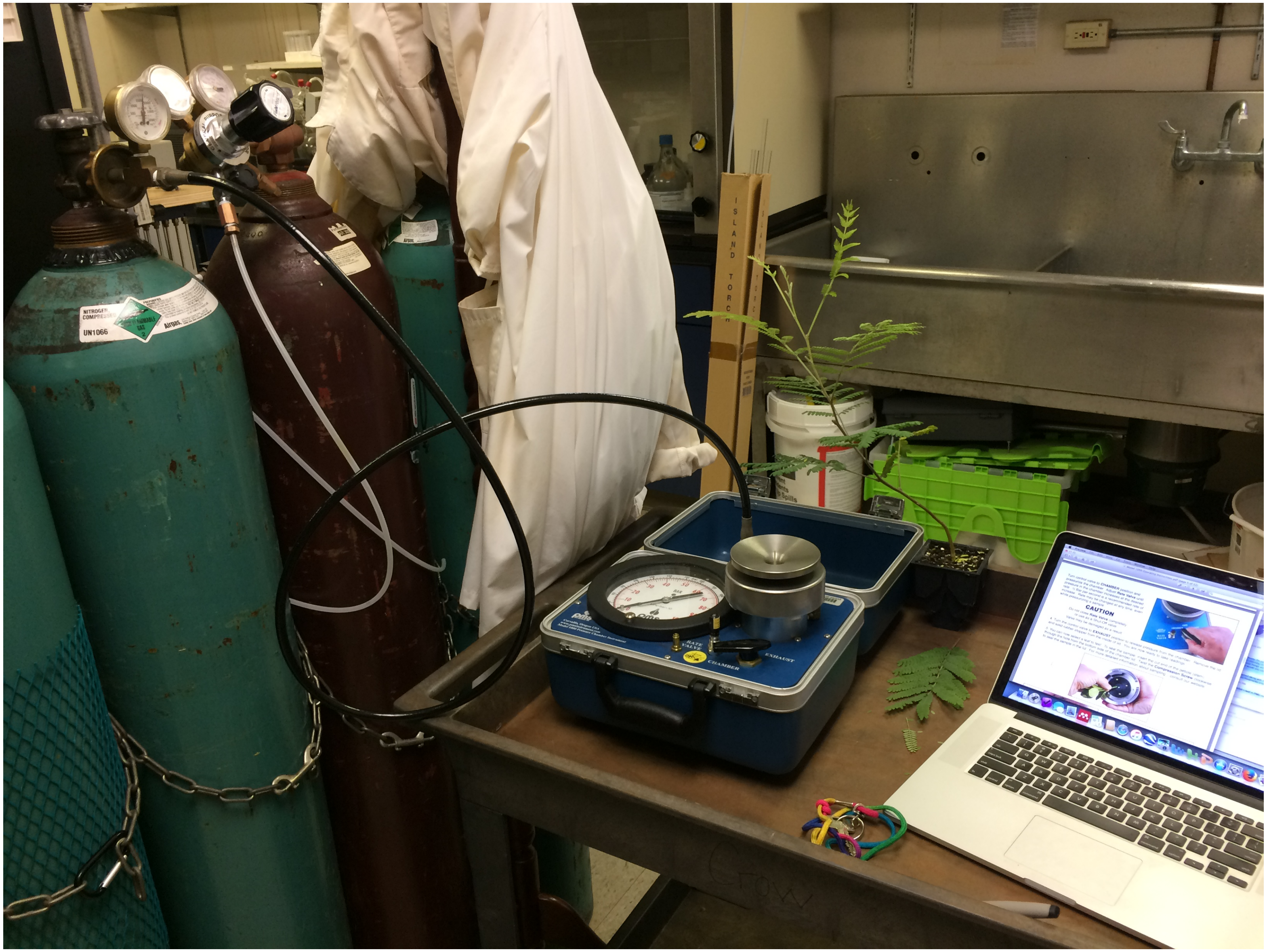


Image 3.4. *Acacia koa* seedlings removed from establishment simulation experiment after 5 weeks of growth post-transplant.

Image 3.5. *Acacia koa* seedlings prior to outplanting in the field study of experiment 2 (human for scale).

Image 3.6. Outplanting study site in the Koʻolau Mountains on the North Shore of Oʻahu.

Image 3.7. *Acacia koa* stocktype produced from Deepot™ D25L and RootMaker® Express™ 18 containers 410 (cm^3).

Image 3.8. Planted *Acacia koa* tree in field study after 8 months of field growth (human for scale).

CHAPTER 4

CONCLUDING REMARKS

This thesis endeavored to evaluate the utility of a reduced Nitrogen hardening regime and its potential as a nursery cultural tool to increase survival in the field. The other aim of this thesis was to assess post-transplant moisture stress, field survival, and growth of two container-types (RootMaker® Express™ 18 and Deepot™ D25L) that offered differing root system modification capabilities.

The first objective of this research, outlined in chapter two, was to investigate if a reduction in the amount of applied nitrogen in the final-phase of nursery culture would increase the root-to-shoot ratio of koa. N hardened seedlings embodied multiple beneficial morphological measures. It was observed that a low-N hardening regime altered the height growth trajectory, and resulted in seedlings having a significantly increased root-to-shoot ratio. While height growth was reduced in N hardened seedlings, root-collar diameter was significantly increased compared to seedlings that received an equal total N without a low-N hardening phase. When the experiment was replicated again in August and October with a greater amount of total N applied, root-to-shoot ratio increases persisted, but to a lesser degree. These findings support previous studies on plant biomass allocation to roots in response to a limited nitrogen supply.

The second objective of this research, outlined in chapter three, was to investigate the effects of a reduced N hardening regime and container-type on post-transplant plant moisture stress. N hardened stocktypes initially exhibited less moisture stress. After 17 days, the main effect of fertilization exhibited no significant effect on mid-day plant moisture stress. N hardened seedlings were under significantly increaseed pre-dawn moisture stress after 12 and 17 days. Upon the completion of the experiment after 17 days, container-type had a highly significant influence on plant moisture stress with Deepot stocktype expressing a significantly reduced measure compared to RootMaker stocktype.

The third objective of this research, outlined in chapter three, was to investigate the effects of a reduced-N hardening regime and container-type on survival and growth of koa outplanted into the field. Survival and growth were assessed at 8 months. A high rate of survival was measured in all treatment combinations (>95%). N hardening conferred no significant benefits or differences in regard to field survival, height growth, and root-collar diameter growth. Container-type had a highly significant effect on *Acacia koa* height and root-collar diameter growth. Deepot stocktype produced trees with a significantly increased height and root-collar diameter in comparison to trees from RootMaker stocktype.

All treatments in the first experiment had a greatly increased RDM and R:S compared to the second and third experiment (Tables 2.3, 3.5, 3.7; Figure 2.3). There are 2 possible explanations for this difference; first, the initial N hardening experiment was conducted in the summer when the light intensity is at its greatest during the year. May to July solar radiation at this location (21°18'09.16"N, 157°48'54.42"W) ranges from 245 to 251 W/m^2. This is a greater intensity compared to September through November with 241 to 158 W/m^2, and November through January with 158 to 156 W/m^2 (Giambelluca 2014). Increased light intensity promotes root growth over shoot growth (Walter and Nagel 2006), and is a probable explanation for the dramatic increase. The second possible explanation is related to seed source, the experiment in chapter 2 utilized seed from Hawai'i Island while the experiments in chapter 3 used seed from O'ahu. Perhaps the different genetics of these seed sources had a role in biomass allocation. It is known from common garden studies and PCR-based phyllogenetic analyses that koa has a tremendous scope of genetic-based phenotypic diversity (Daehler et al. 1999; Adamski et al. 2012).

The increased total applied nitrogen during nursery culture in the field simulation study did not confer a significant increase in RDM or R:S. This is in stark contrast to the experiment in chapter 2 where RDM was increased by 56% in N hardened seedlings compared to seedlings without N hardening with the same total N applied. In the discussion of the first study, the question of N dosage during the rapid-growth phase was posed. Following that experiment, it was hypothesized that the treatment without a

N hardening phase but with an equal total N applied perhaps received a suboptimal dose of applied N which implicated its growth-rate and subsequent biomass allocation. To answer that question, the total target N was increased to 184 mg N in the establishment simulation study and 172 mg N in the field study (rapid-growth phase N mg per application event increased to 7.18 and 5.5, respectively) (Table 3.1, 3.3). The resulting difference in RDM and R:S was reduced in contrast to the results in chapter 2. N hardened RootMaker seedlings in the first experiment experienced a 37% increase in R:S (Figure 2.3). This is a greater increase than in the establishment simulation study (non-significant) and the field study (22%).

While Deepot stocktype outperformed RootMaker stocktype in regard to field height and root-collar diameter growth, it is worth considering that this site's soil profile was ideal for the planting of 26 cm long containers. The rate of growth documented by RootMaker stocktype do not necessarily need to be considered inadequate. RootMaker-grown trees experienced a high survival rate, and sufficient rate of growth. It cannot be ruled-out that the root structure of RootMaker-grown trees is of a high quality in the field due to the preclusion of root-spiraling and constriction in container culture.

Future study of field root egress would be interesting and useful to determine field root architecture differences between stocktype. This would be an exceptionally difficult yet interesting endeavor to undertake. Many of the soils in Hawaii are not as deep or free of stones as this site was. When planting into more shallow soils (as are widely found on Hawai'i Island), RootMaker containers could perhaps have a comparable advantage. These studies would be interesting to replicate on sites with exceptionally wet, dry, and shallow soils to document potential site-related interactions.

Further study of multi-element interactions, nutrient dose optimization, and their influence on familial seed origins would be useful to advance the efficient production of high-quality, field-ready koa seedlings. Identifying and studying other factors that influence the promotion of hardened, field-ready koa seedlings would also be of great importance. Further study of biomass allocation in response to irrigation levels could reveal an optimal seedling irrigation rate, and container dry-down percentage of gravimetric water content.

Optimizing nutrient and irrigation application rates to produce stocktype with beneficial structural and physiological attributes, with a subsequent evaluation of the efficacy of those production methods, tested in multiple outplanting scenarios that encompass multiple establishment constraining factors, would be a tremendous boon to the future of *Acacia koa* and the plants and animals that depend on koa forests. In order to trounce the continuously creeping alien plant invasion that is devastating our uniquely marvelous Hawaiian islands, we must endeavor toward a vast and transformative reforestation effort unlike anything the state of Hawaiʻi has ever seen. The future of the Hawaiian forests are in our hands. Defenseless native plants desperately need our help, and all efforts toward rehabilitation are of a tremendous value to the future of these islands.

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